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1c803 U.S. PTO

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802578/60  
Jc675 U.S. PTO

# UTILITY PATENT APPLICATION TRANSMITTAL

Attorney Docket No. 195378US0DIV

First Inventor or Application Identifier Chieko OSUMI

Title RAFFINOSE SYNTHASE GENE, METHOD OF PRODUCING RAFFINOSE,  
AND TRANSGENIC PLANT

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

1. ☒ Fee Transmittal Form (e.g. PTO/SB/17)  
(Submit an original and a duplicate for fee processing)
2. ☒ Specification Total Pages **41**
3. ☒ Drawing(s) (35 U.S.C. 113) Total Sheets **7**
4. ☒ Oath or Declaration Total Pages **3**
  - a. ☐ Newly executed (original or copy)
  - b. ☒ Copy from a prior application (37 C.F.R. §1.63(d))  
(for continuation/divisional with box 15 completed)
    - i. ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting inventor(s) named  
in the prior application, see 37 C.F.R. §1.63(d)(2) and  
1.33(b).
5. ☒ Incorporation By Reference (usable if box 4B is checked)  
The entire disclosure of the prior application, from which a copy of  
the oath or declaration is supplied under Box 4B, is considered to be  
part of the disclosure of the accompanying application and is hereby  
incorporated by reference therein.

ADDRESS TO: Assistant Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

## ACCOMPANYING APPLICATION PARTS

6. ☒ The prior application is assigned to: Ajinomoto  
Company, Inc., Reel No.: 8778, Frame No. 0242
7. ☐ 37 C.F.R. §3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)
8. ☐ English Translation Document (if applicable)
9. ☐ Information Disclosure  
Statement (IDS)/PTO-1449 ☐ Copies of IDS  
Citations
10. ☒ Preliminary Amendment
11. ☒ White Advance Serial No. Postcard
12. ☐ Small Entity ☐ Statement filed in prior  
Statement(s) application. Status still proper  
and desired.
13. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
14. ☒ Other: Request for Priority  
Certified Statement Re Filing in Foreign Language,  
Verification of Translation, Spec. 83 pp. And 12  
Claims/Drawings 7 filed in prior U.S. Application  
08/864,234, Sequence Listing

15. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below:

☐ Continuation ☒ Divisional ☐ Continuation-in-part (CIP) of prior application no.: 08/846,234  
Prior application information: Examiner: Zaghmout Group Art Unit: 1638

16. Amend the specification by inserting before the first line the sentence:

☒ This application is a ☐ Continuation ☒ Division ☐ Continuation-in-part (CIP)  
of application Serial No. 08/846,234 Filed on April 28, 1997, now allowed.

☐ This application claims priority of provisional application Serial No. Filed

## 17. CORRESPONDENCE ADDRESS



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Signature:		Date:	9/22/00
Name:	James J. Kelly, Ph.D	Registration No.:	41,504

Docket No. 195378US0DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INVENTOR(S) Chieko OSUMI et al.

SERIAL NO: New Application

FILING DATE: Herewith

FOR: RAFFINOSE SYNTHASE GENE, METHOD OF PRODUCING RAFFINOSE, AND TRANSGENIC PLANT

FEE TRANSMITTAL

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

FOR	NUMBER FILED	NUMBER EXTRA	RATE	CALCULATIONS
TOTAL CLAIMS	24 - 20 =	4	× \$18 =	\$72.00
INDEPENDENT CLAIMS	7 - 3 =	4	× \$78 =	\$312.00
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS (If applicable)			+ \$260 =	\$0.00
<input type="checkbox"/> LATE FILING OF DECLARATION			+ \$130 =	\$0.00
BASIC FEE				\$690.00
TOTAL OF ABOVE CALCULATIONS				\$1,074.00
<input type="checkbox"/> REDUCTION BY 50% FOR FILING BY SMALL ENTITY				\$0.00
<input type="checkbox"/> FILING IN NON-ENGLISH LANGUAGE			+ \$130 =	\$0.00
<input type="checkbox"/> RECORDATION OF ASSIGNMENT			+ \$40 =	\$0.00
TOTAL				\$1,074.00

- ☐ Please charge Deposit Account No. 15-0030 in the amount of A duplicate copy of this sheet is enclosed.
- ☒ A check in the amount of **\$1,074.00** to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge any additional fees which may be required for the papers being filed herewith and for which no check is enclosed herewith, or credit any overpayment to Deposit Account No. 15-0030. A duplicate copy of this sheet is enclosed.

Respectfully Submitted,

OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.

Date: 9/25/00

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Docket No. 195378US0DIV

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :  
CHIEKO OSUMI ET AL : ATTN: APPLICATION DIVISION  
SERIAL NO: NEW APPLICATION :  
FILED: HEREWITH :  
FOR: RAFFINOSE SYNTHASE GENE, :  
METHOD FOR PRODUCING :  
RAFFINOSE, AND TRANSGENIC :  
PLANT

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as follows.

Page 1, after the title, please insert:

--This application is a Divisional of U.S. Application Serial No. 08/846,234, filed April 28, 1997, now allowed.--.

Page 10, line 17, replace "57 to 2408" with --56 to 2407--;

line 22, replace "57 to 2408" with --56 to 2407--.

Please delete the original Sequence Listing at pages 66-78.

After the Abstract at page 83, please insert the attached substitute Sequence Listing.

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IN THE CLAIMS

Please cancel Claims 1-12.

Please add the following claims.

--13. An isolated DNA encoding a raffinose synthase having the following properties:

(1) action and substrate specificity: the raffinose synthase produces raffinose from sucrose and galactinol;

(2) optimum pH: the raffinose synthase has an optimum pH of about 6 to 8;

(3) optimum temperature: the raffinose synthase has an optimum temperature of about 35 to 40°C;

(4) molecular weight: the raffinose synthase has:

(i) a molecular weight of about 75 kDa to 95 kDa estimated by gel filtration chromatography;

(ii) a molecular weight of about 90 kDa to 100 kDa estimated by polyacrylamide gel electrophoresis; and

(iii) a molecular weight of about 90 kDa to 100 kDa estimated by SDS-polyacrylamide gel electrophoresis under a reduced condition; and

(5) inhibition: the raffinose synthase is inhibited by iodoacetamide, N-ethylmaleimide, and myo-inositol.

14. The DNA of Claim 13, which wherein the raffinose synthase comprises an amino acid sequence shown in SEQ ID NO: 1, 2 or 3.

15. An isolated DNA encoding a raffinose synthase, wherein the DNA is hybridizable under stringent conditions to a DNA comprising nucleotide numbers 56 to 2407 of SEQ ID NO: 4.

16. The DNA of Claim 15, wherein the stringent conditions are 0.1 x SSC, 0.1% SDS at 60°C.

17. An isolated DNA encoding a raffinose synthase having a homology of not less than 35% with respect to the raffinose synthase shown in SEQ ID NO: 5.

18. The DNA of Claim 17, wherein the raffinose synthase has a homology of not less than 40% with respect to the raffinose synthase shown in SEQ ID NO: 5.

19. The DNA of Claim 17, wherein the raffinose synthase has a homology of 65% in the region between the 510<sup>th</sup> and 610<sup>th</sup> amino acid of SEQ ID NO: 5.

20. An isolated DNA encoding a raffinose synthase, wherein the DNA has a homology of not less than about 50% with respect to the nucleotide sequence of SEQ ID NO: 4.

21. An isolated DNA encoding a raffinose synthase, wherein the DNA has a homology of not less than about 65% with respect to a region comprising about 300 nucleotide residues in the nucleotide sequence of SEQ ID NO: 4.

22. An isolated DNA encoding a raffinose synthase, wherein the DNA is obtained from a dicotyledonous plant.

23. The DNA of Claim 22, wherein the dicotyledonous plant is a *Cucurbitaceae* *Leguminosae* or plant

24. The DNA of Claim 22, wherein the dicotyledonous plant is a *Cucurbitaceae* plant.

25. The DNA of Claim 24, wherein the *Cucurbitaceae* plant is a melon or a cucumber.

26. The DNA of Claim 24, wherein the *Cucurbitaceae* plant is *Cucumis melo* or *Cucumis sativus*.

27. An isolated DNA encoding a raffinose synthase, wherein the DNA is obtained by a process comprising screening a cDNA library isolated from cucumber by hybridization with an oligonucleotide probe, wherein the oligonucleotide probe encodes a partial amino acid sequence of SEQ ID NO: 5.

28. The DNA of Claim 15, wherein the raffinose synthase has a homology of not less than 35% with respect to the raffinose synthase shown in SEQ ID NO: 5.

29. The DNA of Claim 15, wherein the raffinose synthase has a homology of not less than 40% with respect to the raffinose synthase shown in SEQ ID NO: 5.

30. The DNA of Claim 15, wherein the raffinose synthase has a homology of not less than 65% in the region between the 510<sup>th</sup> and 610<sup>th</sup> amino acid of SEQ ID NO: 5.

31. The DNA of Claim 13, wherein the DNA is hybridizable under stringent conditions to a DNA comprising nucleotide numbers 56 to 2407 of SEQ ID NO: 4.

32. The DNA of Claim 13, wherein the stringent conditions are 1 x SSC, 0.1% SDS at 60°C.

33. The DNA of Claim 13, wherein the stringent conditions are 0.1 x SSC, 0.1% SDS at 60°C.

34. The DNA of Claim 13, wherein the raffinose synthase has a homology of not less than 35% with respect to the raffinose synthase shown in SEQ ID NO: 5.

35. The DNA of Claim 13, wherein the raffinose synthase has a homology of not less than 40% with respect to the raffinose synthase shown in SEQ ID NO: 5.

36. The DNA of Claim 13, wherein the raffinose synthase has a homology of not less than 65% in the region between the 510<sup>th</sup> and 610<sup>th</sup> amino acid of SEQ ID NO: 5.--

#### SUPPORT FOR THE AMENDMENTS

Page 1 of the specification has been amended to insert a reference to the parent application. Page 10 of the specification has been amended to correct an error identifying the coding sequence in SEQ ID NO: 4. This correction is supported by the entry for SEQ ID NO: 4 in the original Sequence Listing at page 67. Newly added Claims 13-36 are supported by the specification at pages 7-76 and by original Claims 1-82. Support for the recitation of dicotyledenous plant in Claim 22 is provided by the fact that the plant disclosed in the present application are dicotyledonous. Support for the recitation of *Leguminosae* in Claim 23 is provided from the Description of the Background Art in the specification at pages 1-7, which clarifies that the dicotyledonous plant such as the *Leguminosae* plant is useable like the Cucurbitaceae plant. No new matter is believed to have been added to this application by these amendments.

#### REMARKS

Claims 13-36 are active in this application.

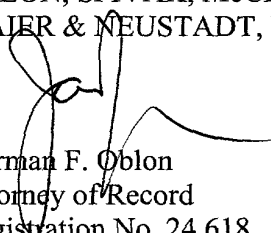
This application is a Divisional of U.S. Application Serial No. 08/846,234, filed April 28, 1997, now allowed.

A substitute Sequence Listing has been submitted. In lieu of submission of a corresponding computer-readable Sequence Listing, Applicants request that the Office use the CRF filed in the parent application on November 15, 1999. Applicants confirm that the sequence information in the substitute Sequence Listing submitted herewith is the same as the CRF submitted on November 15, 1999 in the parent application.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,  
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Chieko OSUMI, et al.

Serial No. 08/846,234

Filed: April 28, 1997

For: RAFFINOSE SYNTHASE GENE, METHOD FOR PRODUCING  
RAFFINOSE, AND TRANSGENIC PLANT

VERIFICATION OF TRANSLATION

Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Yoshiyuki KAWAGUCHI, of c/o TOYAMA, MATSUKURA,  
KAWAGUCHI & ONO, Yokoyama Building 6th Floor, 4-10, Higashi  
Nihonbashi 3-chome, Chuo-ku, Tokyo, 103 Japan, declare:-

- (1) that I know well both Japanese and English languages;
- (2) that I translated the attached Text of Specification  
and Claims from Japanese into English;
- (3) that the attached English translation is a true and  
correct translation of the Japanese text of specification and  
claims as filed in the United States Patent and Trademark  
Office on April 28, 1997 under Serial No. 08/846,234 to the  
best of my knowledge and belief; and
- (4) that all statements made of my own knowledge are true  
and that all statements made on information and belief are  
believed to be true, and further that these statements are  
made with the knowledge that willful false statements and the  
like are punishable by fine or imprisonment, or both, under 18  
USC 1001, and that such false statements may jeopardize the  
validity of the application or any patent issuing thereon.

Signed at Tokyo, Japan, this 1st day of September, 1997.

  
\_\_\_\_\_  
Yoshiyuki KAWAGUCHI

RAFFINOSE SYNTHASE GENE, METHOD FOR PRODUCING RAFFINOSE,  
AND TRANSGENIC PLANT

Technical Field

0052508-092900

The present invention relates to a raffinose  
5 synthase, a method for raffinose synthesis based on the  
use of raffinose synthase or a cell-free extract  
containing the raffinose synthase, DNA coding for the  
raffinose synthase, and methods for its use to produce  
altered amount of raffinose family oligosaccharides in  
10 transformed plants. Raffinose is utilized in a variety  
of fields, as a food material having an activity to  
proliferate Bifidobacterium, or as a pharmaceutical to  
be used, for example, for solutions of organ  
preservation.

15 Background Art

Raffinose is one of raffinose family  
oligosaccharides, in which galactose is connected to  
glucosyl group of sucrose via  $\alpha$ -1,6 linkage. The  
raffinose family oligosaccharides include, for example,  
20 stachyose containing two connected galactose residues,  
and verbascose containing three connected galactose  
residues, in addition to raffinose. These  
oligosaccharides are widely distributed in plants, for

example, seeds of various plants such as beans, rapeseed, and cottonseed containing these oligosaccharides as reserve carbohydrates; plants belonging to Cucurbitaceae such as cucumber and melon  
5 containing these sugars as transport sugars; and sugar beet (Beta vulgaris) and rosette leaves having acquired cold resistance.

The raffinose family oligosaccharides are biosynthesized as follows.

- 10 UDP-galactose + myo-inositol → galactinol + UDP ... (a)  
galactinol + sucrose → raffinose + myo-inositol ... (b)  
galactinol + raffinose → stachyose + myo-inositol ... (c)

The respective reactions are catalyzed by (a) galactinol synthase (GS: EC 2.4.1.123), (b) raffinose  
15 synthase (RS: EC 2.4.1.82), and (c) stachyose synthase (STS: EC 2.4.1.67).

At present, raffinose is extracted from sugar beet, and it is separated and purified in the sucrose purification process. However, since crystal formation  
20 of sucrose is deteriorated by raffinose, sugar beet has been subjected to breeding and improvement with the aim of decreasing the raffinose content. As a result, the raffinose content in sugar beet now has a low value of 0.03 % to 0.16 % (Enzyme Microb. Technol., Vol. 4, May,  
25 130-135 (1982)). Therefore, it is not easy to efficiently obtain raffinose from sugar beet having such a low raffinose content.

As described above, raffinose is contained in mature seeds of plants belonging to Leguminosae represented by soybean. Mature seed of soybean contains, as soybean oligosaccharides, sucrose (content: about 5 %), stachyose (content: about 4 %), and raffinose (content: about 1 %). The soybean oligosaccharides are recovered in a fraction obtained by deproteinizing defatted soybean, and they are utilized, for example, for functional food products after concentration. However, raffinose occupies a proportion of 10 % of the whole oligosaccharides, and hence raffinose exists in a small amount.

On the other hand, a method for enzymatically synthesizing raffinose has been reported (Trends in Glycoscience and Glycotechnology, 7.34, 149-158 (1995)). This method comprises the steps of synthesizing galactobiose in accordance with a condensation reaction catalyzed by  $\alpha$ -galactosidase, and transferring galactosyl group to sucrose by using the galactobiose as a galactosyl group donor in accordance with a galactosyl transfer reaction to synthesize raffinose. However, in this reaction, 350 g of galactobiose is synthesized from 1.9 kg of lactose hydrolysate, and 100 g of raffinose is obtained from 190 g of galactobiose and 760 g of sucrose. Therefore, the yield of produced raffinose is low, and hence this synthesis method is not efficient.

Besides the foregoing methods, a method is also

conceivable in which a plant having a high raffinose content may be bred by means of transformation for genes for enzymes included in the biosynthesis system. For example, Kerr et al. have cloned a gene for galactinol synthase, and transformed rapeseed therewith (WO 93/02196). As a result, the GS activity was increased, however, the content of the raffinose family oligosaccharides was unwillingly decreased. It was impossible to achieve the object to enhance the biosynthesis of the raffinose family oligosaccharides by transforming the galactinol synthase gene. Therefore, there has not been provided a method for increasing the content of the raffinose family oligosaccharides in plant.

On the other hand, it is also demanded to decrease the raffinose family oligosaccharides. As described above, the raffinose family oligosaccharides are widely distributed over plants including, seeds of various plants such as beans, for example, soybean, rapeseed, and cottonseed containing these oligosaccharides as storage carbohydrates; plants belonging to Cucurbitaceae such as cucumber and melon containing these oligosaccharides as transport sugars; and sugar beet and rosette leaves having acquired cold resistance. Meals obtained after extraction of oil, for example, from soybean, rapeseed, and cotton contain the raffinose family oligosaccharides. Almost all of the meals are

utilized as feed. However, human and animals, which do not have  $\alpha$ -galactosidase, cannot directly digest the raffinose family oligosaccharides. It is known that the raffinose family oligosaccharides lower the metabolic energy efficiency of feed due to, for example, assimilation of the raffinose family oligosaccharides by enteric bacteria to cause gas production. It has been reported that removal of raffinose family oligosaccharides from soybean meal results in a large increase in the metabolizable energy for broiler chickens (Coon, "Proceeding Soybean Utilization Alternatives", University of Minnesota, 203-211 (1989)). In view of the foregoing facts, it is desired to develop the plants such as soybean, rapeseed, and cottonseed in which the raffinose family oligosaccharides are decreased.

Such plants have been subjected to breeding to increase the amount of oil. Photosynthetic products are distributed over oils, proteins, and carbohydrates including the raffinose family oligosaccharides. It has been reported for soybean that a reverse correlation exists between the amount of oils and the amount of carbohydrates. It is expected that the content of oils can be increased in a soybean plant having the same photosynthetic ability as those possessed by others, by decreasing the production of the raffinose family oligosaccharides.

Based on a viewpoint as described above, Kerr et al. have reported development of soybean varieties with a low content of the raffinose family oligosaccharides, by means of breeding based on mating and selection, in which the raffinose family oligosaccharides are lowered by an amount of 80 % to 90 % (WO 93/00742). However, this technique concerns creation of soybean variety, which cannot be applied to other various soybean varieties developed in response to, for example, aptitude for cultivation and resistance to disease. This technique cannot be universally applied to various plants as well.

It is known that raffinose, which is contained, for example, in sugar beet and sugar cane, lowers crystal formation of sugar or sucrose. Therefore, it is possible to expect that if no raffinose is produced, the production efficiency of sugar may be improved in such a plant. However, no sugar beet has been created, which contains no raffinose.

As described above, the raffinose synthase, which has been hitherto purified, has been confirmed only as an enzyme activity, and no entity of the enzyme has been identified. The confirmed activity is low, and it has been desired to obtain a raffinose synthase having a high activity. The conventional method for producing raffinose provides a low yield, and hence it has been desired to develop an efficient method for producing

raffinose. On the other hand, it is also desired to breed a plant in which the raffinose family oligosaccharides are decreased.

### Disclosure of the Invention

5           The present invention has been made taking the foregoing viewpoints into consideration, an object of which is to obtain a raffinose synthase having a high activity and DNA encoding raffinose synthase, and provide an efficient method for enzymatically synthesis  
10 raffinose, and a method for utilizing DNA encoding raffinose synthase in plants.

          As a result of diligent investigations in order to achieve the object described above, the present inventors have succeeded in purifying a raffinose  
15 synthase from cucumber. Further diligent investigations have been made by the present inventors in order to clone a gene coding for the raffinose synthase. As a result, a DNA fragment specific to a gene for the raffinose synthase has been obtained by chemically  
20 synthesizing single strand DNA's on the basis of nucleotide sequences deduced from amino acid sequences of peptide fragments of the cucumber raffinose synthase, and performing PCR by using the single strand synthetic DNA's as primers and using cDNA's prepared from  
25 poly(A)'RNA extracted from cucumber as templates.

Further, the raffinose synthase gene has been isolated by adopting a method in which hybridization is performed for a cDNA library originating from cucumber by using the DNA fragment as a probe. A chimeric gene having a regulatory region expressible in plants has been prepared by using a fragment of the isolated raffinose synthase gene to transform a plant. Further, the function of endogenous raffinose synthase has been regulated by introducing the raffinose synthase gene to create a plant in which the raffinose family oligosaccharides are decreased.

Namely, the present invention provides a raffinose synthase which has the following properties:

(1) action and substrate specificity: the raffinose synthase produces raffinose from sucrose and galactinol;

(2) optimum pH: the raffinose synthase has an optimum pH of about 6 to 8;

(3) optimum temperature: the raffinose synthase has an optimum temperature of about 35 to 40 °C;

(4) molecular weight: the raffinose synthase has:

(i) a molecular weight of about 75 kDa to 95 kDa estimated by gel filtration chromatography;

(ii) a molecular weight of about 90 kDa to 100 kDa estimated by polyacrylamide gel electrophoresis (Native PAGE); and

(iii) a molecular weight of about 90 kDa to 100 kDa

estimated by SDS-polyacrylamide gel electrophoresis  
(SDS-PAGE) under a reduced condition;

(5) inhibition: the raffinose synthase is  
inhibited by iodoacetamide, N-ethylmaleimide, and myo-  
5 inositol.

In a specified embodiment of the foregoing  
raffinose synthase provided by the present invention,  
the raffinose synthase has an amino acid sequence  
including respective amino acid sequences shown in SEQ  
10 ID NOs. 1 to 3 in Sequence Listing.

In another aspect of the present invention, there  
is provided a raffinose synthase which is a protein  
specified by the following item (A) or (B):

(A) a protein which has an amino acid sequence  
15 shown in SEQ ID NO: 5 in Sequence Listing; or

(B) a protein which comprises an amino acid  
sequence including substitution, deletion, insertion,  
addition, or inversion of one or several residues of  
amino acids in the amino acid sequence shown in SEQ ID  
20 NO: 5 in Sequence Listing, and which has an activity to  
produce raffinose from sucrose and galactinol.

In still another aspect of the present invention,  
there is provided a method for producing raffinose,  
comprising the step of allowing the foregoing raffinose  
25 synthase to act on sucrose and galactinol to produce  
raffinose.

In still another aspect of the present invention,

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there are provided DNA encoding raffinose synthase, and DNA coding for a protein specified by the following item

(A) or (B):

(A) a protein which has an amino acid sequence  
5 shown in SEQ ID NO: 5 in Sequence Listing; or

(B) a protein which comprises an amino acid  
sequence including substitution, deletion, insertion,  
addition, or inversion of one or several residues of  
amino acids in the amino acid sequence shown in SEQ ID  
10 NO: 5 in Sequence Listing, and which has an activity to  
produce raffinose from sucrose and galactinol.

In a specified embodiment of the foregoing DNA of  
the present invention, there is provided DNA specified  
by the following item (a) or (b):

15 (a) DNA which includes a nucleotide sequence  
comprising at least nucleotide residues having  
nucleotide numbers of 57 to 2408 in a nucleotide  
sequence shown in SEQ ID NO: 4 in Sequence Listing; or

(b) DNA which is hybridizable under a stringent  
20 condition with the nucleotide sequence comprising at  
least nucleotide residues having nucleotide numbers of  
57 to 2408 in the nucleotide sequence shown in SEQ ID  
NO: 4 in Sequence Listing, and which codes for a protein  
having an activity to produce raffinose from sucrose and  
25 galactinol.

In still another aspect of the present invention,  
there are provided a chimeric gene comprising a

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raffinose synthase gene or a part thereof, and a transcription regulatory region expressible in plant cells, and a plant transformed with the chimeric gene.

In still another aspect of the present invention,  
5 there is provided a method for changing a content of raffinose family oligosaccharides in a plant, comprising the steps of transforming the plant with the chimeric gene, and expressing the gene in the plant.

In the following description, the raffinose  
10 synthase having the properties described in the foregoing items (1) to (5), or the raffinose synthase specified as the protein defined in the foregoing items (A) and (B) is simply referred to as "raffinose synthase" in some cases. DNA encoding raffinose  
15 synthase, or DNA encoding raffinose synthase and including non-translating regions is referred to as "raffinose synthase gene" in some cases.

The present invention will be explained in detail below.

20 <1> Raffinose synthase of the present invention

The raffinose synthase of the present invention has the following properties:

(1) action and substrate specificity: the raffinose synthase produces raffinose from sucrose and  
25 galactinol;

(2) optimum pH: the raffinose synthase has an

optimum pH of about 6 to 8;

(3) optimum temperature: the raffinose synthase has an optimum temperature of about 35 to 40 °C;

(4) molecular weight: the raffinose synthase has:

5 (i) a molecular weight of about 75 kDa to 95 kDa estimated by gel filtration chromatography;

(ii) a molecular weight of about 90 kDa to 100 kDa estimated by polyacrylamide gel electrophoresis (Native PAGE); and

10 (iii) a molecular weight of about 90 kDa to 100 kDa estimated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under a reduced condition;

(5) inhibition: the raffinose synthase is inhibited by iodoacetamide, N-ethylmaleimide, and myo-  
15 inositol.

The raffinose synthase having the foregoing properties has been isolated and purified from leaves of cucumber, which has been identified for the first time by the present inventors. As demonstrated in Examples  
20 described later on, the raffinose synthase originating from cucumber includes the respective amino acid residues shown in SEQ ID NOs: 1 to 3 in Sequence Listing, in the amino acid sequence of the enzyme protein. An entire amino acid sequence of the raffinose  
25 synthase is shown in SEQ ID NO: 5.

The raffinose synthase is obtainable from plants belonging to Cucurbitaceae, for example, plants such as

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melon (Cucumis melo) and cucumber (Cucumis sativus). Especially, the raffinose synthase is contained in a large amount in leaves of these plants, especially in tissues of leaf veins and seeds.

5           Next, the method for producing the raffinose synthase of the present invention will be explained in accordance with an illustrative method for isolating and purifying the raffinose synthase from cucumber.

10           Leaf vein tissues are collected from leaves of cucumber obtained 6 to 10 weeks after planting. The vein are ground by a mortar with liquid nitrogen, to which a buffer is added to extract proteins. During this process, it is allowable to add a substance to prevent the raffinose synthase from degradation and  
15           inactivation, for example, a protease inhibitor such as PMSF (phenylmethanesulfonyl fluoride), or polyclarl AT (produced by Serva). Insoluble matters are removed from an obtained extract solution by means of filtration and centrifugation to obtain a crude extract solution.

20           The crude extract solution thus obtained is subjected to fractionation based on combination of ordinary methods for purifying proteins, including, for example, anion exchange chromatography, hydroxyapatite chromatography, gel filtration, and salting out. Thus  
25           the raffinose synthase can be purified.

          Anion exchange chromatography can be performed, for example, by using a column charged with a strongly basic

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anion exchanger such as HiTrap Q (produced by Pharmacia), or a weakly basic anion exchanger such as DEAE-TOYOPEARL (produced by Toyo Soda). The extract solution containing the raffinose synthase is allowed to pass through the column so that the enzyme is adsorbed to the column. After washing the column, the enzyme is eluted by using a buffer having a high salt concentration. During this process, the salt concentration may be increased in a stepwise manner, or the concentration gradient may be applied. For example, when the HiTrap Q column is used, the raffinose synthase activity adsorbed to the column is eluted by NaCl at about 0.3 M. An eluting solution to give an NaCl concentration gradient of 0.05 M to 0.35 M is preferably used for DEAE-TOYOPEARL. An eluting solution to give a phosphate concentration gradient of 0.01 M to 0.3 M is preferably used for hydroxyapatite chromatography.

The order of the foregoing operations is not specifically limited. Each of the operations may be repeated two or more times. It is desirable to exchange a sample solution with an appropriate buffer by means of dialysis or the like before the sample solution is allowed to pass through each column. The sample solution may be concentrated at each stage.

At each stage of the purification, it is preferable that the raffinose synthase activity contained in each of fractionated fractions is measured so that fractions

having high activities are collected to be used in the next stage. The method for measuring the raffinose synthase activity is exemplified by a method based on the use of radioisotope as reported, for example, by

5    Lehle, H. et al. (Eur. J. Biochem., 38, 103-110 (1973)).

As a modified method thereof, the reaction temperature and the substrate concentration may be changed. For example, 10 µl of an enzyme solution is added to a reaction solution containing, at final concentrations,

10    10 mM <sup>14</sup>C-sucrose, 20 mM galactinol, 25 mM HEPES (2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulfonic acid)-NaOH, pH 7.0, 0.5 mM DTT (dithiothreitol) to give a volume of 50 µl. The solution is incubated at 32 °C for 1 hour to perform the reaction. The reaction is stopped

15    by adding 200 µl of ethanol and heating the solution at 95 °C for 30 seconds. The reaction solution is centrifuged to obtain a supernatant. An aliquot of the supernatant is spotted on Whatman 3MM filter paper, and developed with n-propanol: ethyl acetate: water = 4:1:2.

20    Incorporation of <sup>14</sup>C into raffinose is investigated, which is regarded to be the raffinose synthase activity (nmol/hour).

The present inventors have developed a method for measuring the raffinose synthase activity in place of

25    the foregoing method. Namely, the raffinose synthase activity is measured by quantitatively determining raffinose produced by the raffinose synthesis reaction,

by means of HPLC (high-performance liquid chromatography). According to this method, the activity can be measured conveniently and quickly as compared with the method of Lehle, H. et al. This method is especially preferable to detect active fractions during the purification operation. This method will be explained below.

The raffinose synthesis reaction is based on the use of a reaction solution prepared to have a composition having the following final concentrations. The reaction solution is added with 10 to 50  $\mu$ l of a raffinose synthase solution to give a volume of 100  $\mu$ l, followed by performing the reaction at 32 °C for 60 minutes.

[Composition of reaction solution (final concentration)]

2.5 mM sucrose  
5 mM galactinol  
5 mM DTT  
20 mM Tris-HCl buffer (pH 7.0)

After performing the reaction as described above, the reaction solution is added with ethanol in a volume four times the volume of the reaction solution to stop the reaction by heating the solution at 95 °C for 30 seconds. The obtained solution is centrifuged to obtain a supernatant which is then dried up under a reduced

pressure. After that, an obtained residue is dissolved in distilled water. Raffinose in the reaction product is quantitatively determined by using HPLC to estimate the raffinose synthase activity. HPLC can be performed  
5 by using, for example, Sugar Analysis System DX500 (CarboPac PA1 column, pulsed amperometry detector (produced by Dionece)).

Fig. 1 shows a result of measurement performed in accordance with the method described above, for the  
10 amount of raffinose produced when the reaction time was changed. As clarified from Fig. 1, this method makes it possible to conveniently measure the raffinose synthase activity with excellent linearity.

The degree of purification of the purified  
15 raffinose synthase can be confirmed, and the molecular weight can be measured, by means of, for example, gel electrophoresis and gel filtration chromatography. Enzymatic properties can be investigated by measuring the enzyme activity while changing the reaction  
20 temperature or the reaction pH, or by measuring the remaining enzyme activity after adding, to the reaction solution, various enzyme inhibitors, metal ions or the like. The stable pH range and the stable temperature range can be investigated by measuring the enzyme  
25 activity after exposing the raffinose synthase to various pH conditions and temperature conditions for a certain period of time respectively.

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The properties of the raffinose synthase described above have been determined in accordance with procedures as described above. However, it should be noted that different results may be obtained depending on measurement conditions. For example, the measurement for the molecular weight based on the use of gel filtration chromatography is affected by the type of the gel filtration agent and the buffer, and the molecular weight marker to be used. The enzyme activity differs depending on the type of the buffer and the salt concentration in many cases even when the measurement is performed at an identical pH. Therefore, upon identification for the raffinose synthase, it is preferable to perform comprehensive investigation without being bound to only measurement for individual properties.

The raffinose synthase of the present invention is obtained by performing the isolation and purification from cucumber as described above. Alternatively, the raffinose synthase of the present invention can be produced by introducing, into an appropriate host, DNA coding for the raffinose synthase described later on, and making expression thereof, in accordance with ordinary methods used for fermentative production of heterogeneous proteins.

Those assumed as the host for expressing the raffinose synthase gene include various procaryotic

cells represented by Escherichia coli, and various eucaryotic cells represented by Saccharomyces cerevisiae. However, it is desirable to use plant cells, especially cells originating from plants such as tobacco, cucumber, and Arabidopsis thaliana.

The recombinant plasmid used for transformation can be prepared by inserting DNA coding for the raffinose synthase into an expression vector in conformity with the type of cells to be used for expression therein. Those usable as the plant expression vector include those having a promoter DNA sequence capable of being expressed in the plant or a combination of a plurality of such promoter DNA sequences, and a terminator DNA sequence workable in the plant, and further having a sequence between the both to make it possible to insert a foreign gene.

The promoter includes, for example, promoters which make expression over a whole plant, such as CaMV 35S RNA promoter, CaMV 19S RNA promoter, and nopaline synthase promoter; promoters which make expression in green tissues, such as Rubisco small subunit promoter; and promoters which make site-specific expression at portions such as seed, including, for example, those for genes of napin and phaseolin. The terminator described above includes, for example, nopaline synthase terminator, and Rubisco small subunit 3'-side portion.

As for the expression vector for plants, for

example, pBI121 and p35S-GFP (produced by CLONTECH) are commercially available, which may be used.

Alternatively, a vector for expressing virus RNA may be used so that a gene for an outer coat protein encoded  
5 thereby, for example, may be replaced with the raffinose synthase gene.

In order to achieve transformation, it is advantageous to use methods which are usually used for transformation, such as the Agrobacterium method, the  
10 particle gun method, the electroporation method, and the PEG method, in conformity with a host cell to be manipulated. The raffinose synthase activity can be detected by using the method adopted in the purification process for the raffinose synthase. Upon the detection,  
15 it is desirable to previously remove  $\alpha$ -galactosidase, for example, by allowing the sample to pass through an anion exchange column.

The gene coding for the raffinose synthase originating from cucumber includes all of those which  
20 provide the raffinose synthase activity upon expression. Preferably, the gene is exemplified by the gene comprising DNA coding for the amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing, and the gene having the nucleotide sequence shown in SEQ ID NO: 4 in  
25 Sequence Listing. It is noted that the gene coding for the amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing includes various nucleotide sequences

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taking degeneracy of codons into consideration. Namely,  
the gene coding for the amino acid sequence shown in SEQ  
ID NO: 5 in Sequence Listing may be selected from such  
various nucleotide sequences, while considering several  
5 factors for the gene expression system, such as  
preferential codons depending on, for example, the type  
of the host cell, and avoidance of higher-order  
structure to be formed by transcribed RNA. The selected  
nucleotide sequence may be DNA cloned from the nature,  
10 or DNA chemically synthesized in an artificial manner.

<2> DNA coding for raffinose synthase of the present  
invention

DNA coding for the raffinose synthase can be  
obtained by preparing a cDNA library from poly(A)<sup>+</sup>RNA  
15 isolated from a plant such as cucumber, and screening  
the cDNA library by means of hybridization. A probe to  
be used for the hybridization can be obtained by  
performing amplification by means of PCR (polymerase  
chain reaction) by using, as primers, oligonucleotides  
20 synthesized on the basis of partial amino acid sequences  
of the raffinose synthase protein.

A method for obtaining DNA of the present invention  
from poly(A)<sup>+</sup>RNA originating from cucumber will be  
specifically explained below.

25 As for the position for extracting poly(A)<sup>+</sup>RNA,  
all portions of a cucumber plant body may be used

provided that the raffinose synthase gene is expressed at that portion. poly(A)<sup>+</sup>RNA can be obtained, for example, from leaves, stalks, buds, fruits, and seeds at various growth stages. However, poly(A)<sup>+</sup>RNA is

5 desirably obtained from a material of fully expanded leaves after fruiting, especially leaf vein portions.

In order to extract total RNA from the cucumber tissue, any method may be used without limitation provided that RNA can be efficiently obtained with less

10 damage. It is possible to use any known method such as the phenol/SDS method and the guanidine isothiocyanate/cesium chloride method. Poly(A)<sup>+</sup>RNA can be isolated from the total RNA thus obtained, by using an oligo(dT) cellulose. It is also preferable to use a

15 kit (for example, MPG Direct mRNA Purification Kit, produced by CPG, INC.) which makes it possible to obtain poly(A)<sup>+</sup>RNA without extracting the total RNA.

A DNA fragment, which is used as a probe for screening for the cDNA library, can be obtained by

20 performing PCR. Oligonucleotides, which have nucleotide sequences deduced from already known amino acid sequences of peptide fragments, for example, nucleotide sequences deduced from amino acid sequences shown in SEQ ID NOs: 1 to 3, are chemically synthesized. The

25 obtained oligonucleotides are used as primers to perform PCR. Any portion of the amino acid sequence of the obtained peptide fragment may be used for the primers.

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However, it is desirable to select sequences which include less degeneracy of codons and which are assumed to form no complicated higher-order structure.

Alternatively, it is also preferable to perform RACE

5 (Rapid Amplification of cDNA End, "PCR PROTOCOLS A Guide to Methods and Applications", ACADEMIC press INC., pp. 28 to 38).

It is desirable to use, as a template for PCR, a cDNA library or single strand cDNA. When heat-resistant  
10 DNA polymerase having a reverse transcriptase activity is used for the PCR reaction, it is allowable to use poly(A)'RNA, or total RNA in some cases.

In order to prepare the cDNA library, at first single strand cDNA's are synthesized by using reverse  
15 transcriptase while using poly(A)'RNA as a template and using oligo(dT) primer and random primers. Next, double strand cDNA's are synthesized in accordance with, for example, the Gubler and Hoffman method, the Okayama-Berg method ("Molecular Cloning", 2nd edition, Cold Spring  
20 Harbor press, 1989). When the raffinose synthase gene is expressed in a small amount, cDNA's may be amplified by means of PCR by using a cDNA library construction kit based on the use of PCR (for example, Capfinder PCR cDNA Library Construction Kit (produced by CLONTECH)).

25 cDNA's thus synthesized can be cloned into a cloning vector such as phage vectors and plasmids, after performing, for example, blunt end formation, addition

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of linker, addition of restriction enzyme site by means of PCR.

5 A portion characteristic of the raffinose synthase cDNA is selected from the DNA fragments obtained by PCR described above, for the probe for hybridization. It is desirable to select a DNA fragment located near to the 5'-terminal side. The amplified DNA fragment thus selected is purified from a reaction solution of PCR. In this procedure, the amplified DNA fragment may be  
10 purified by subcloning the DNA fragment by using a plasmid, preparing a large amount of a subcloned plasmid which is thereafter digested with a restriction enzyme, and excising the DNA fragment from a gel after electrophoresis. Alternatively, the amplified DNA  
15 fragment may be purified by performing PCR by using the plasmid as a template to amplify and use only the objective portion. When the amount of the initially amplified DNA fragment is sufficiently large, the amplified DNA fragment may be purified by  
20 electrophoresing the DNA fragment without performing subcloning, excising a gel segment containing a band of the objective DNA fragment, and purifying the DNA fragment from the gel segment.

Screening to obtain the objective clone from the  
25 cDNA library is performed by means of hybridization. The DNA fragment obtained in accordance with the foregoing method can be labeled and used as a probe for

the hybridization. Upon labeling, it is possible to use various labels such as radioisotope and biotin.

However, labeling is desirably performed in accordance with the random priming method. Screening may be

5 performed by using PCR instead of hybridization.

Further, screening may be performed by using hybridization and PCR in combination.

The nucleotide sequence of DNA coding for the raffinose synthase originating from cucumber obtained as  
10 described above, and the amino acid sequence deduced from the nucleotide sequence are illustratively shown in SEQ ID NO: 4 in Sequence Listing. Only the amino acid sequence is shown in SEQ ID NO: 5. A transformant  
15 AJ13263 of Escherichia coli JM109, which harbors a plasmid pMossloxCRS containing the DNA fragment including DNA coding for the raffinose synthase obtained in Example 3 described later on, has been  
internationally deposited on the basis of the Budapest Treaty since November 19, 1996 in National Institute of  
20 Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3 Higashi-Icchome, Tsukuba-shi, Ibaraki-ken, Japan), and awarded a deposition number of FERM BP-5748.

25 The DNA of the present invention may code for a raffinose synthase protein including substitution, deletion, insertion, addition, or inversion of one or

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several residues of amino acids at one or several positions, provided that the activity of raffinose synthase encoded thereby, i.e., the activity to produce raffinose from sucrose and galactinol is not

5 deteriorated. In this context, the number of "several residues" differs depending on the position and the type of the amino acid residues in the three-dimensional structure of the protein, originally because of the following reason. Namely, high similarity is found

10 between some amino acids and other amino acids, for example, between isoleucine and valine, and such a difference in amino acid does not greatly affect the three-dimensional structure of the protein. Therefore, the DNA of the present invention may code for those

15 having homology of not less than 35 to 40 % with respect to the entire 784 amino acid residues for constructing the raffinose synthase originating from cucumber, provided that they have the raffinose synthase activity. Preferably, those encoded by the DNA of the present

20 invention have homology of 65 % in a region between 510th amino acid and 610th amino acid. Specifically, the number of "several residues" is 2 to 40, preferably 2 to 20, and more preferably 2 to 10.

The present invention includes genes in which

25 homology of not less than about 50 % is given for the entire length of the gene, and homology of not less than 65 % is given over a region comprising about 300

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nucleotide residues. Nucleotide sequence information on such genes can be obtained by searching genes having homology to the raffinose synthase gene originating from cucumber, by using data base such as GenBank. For example, GENETIX-MAC (software for processing genetic information, produced by Software Development), which adopts the Lipman-Person method, may be used as a program for homology analysis. Alternatively, those open to the public on the Internet may be used for this purpose. Some nucleotides sequences obtained by the method as described above contain the entire length of the gene, and other nucleotide sequences do not contain the entire length of the gene. When the entire length of the gene is not contained, the entire length gene can be easily obtained by using RNA extracted from an objective plant tissue as a template, and using primers corresponding to portions having high homology to the raffinose synthase gene originating from cucumber, in accordance with the 5'-RACE method and the 3'-RACE method. The obtained entire length gene may be incorporated into an appropriate expression vector provided as those included in a kit such as Soluble Protein Expression System (produced by INVITROGEN), Tight Control Expression System (produced by INVITROGEN), and QIAexpress System (produced by QIAGEN) so that the gene may be expressed. The raffinose synthase activity may be measured in accordance with the

method described above to select a clone having the activity.

DNA, which codes for substantially the same protein as the raffinose synthase, is obtained by modifying the nucleotide sequence in accordance with, for example, the site-directed mutagenesis method so that amino acids located at specified positions are subjected to substitution, deletion, insertion, or addition. Modified DAN as described above may be also obtained in accordance with the conventionally known mutation treatment. The mutation treatment includes a method in which the DNA coding for the raffinose synthase is treated with hydroxylamine or the like in vitro, and a method in which a bacterium belonging to the genus Escherichia harboring the DNA coding for the raffinose synthase gene is treated with ultraviolet irradiation or a mutating agent usually used for artificial mutation, such as nitrous acid and N-methyl-N'-nitro-N-nitrosoguanidine (NTG).

The substitution, deletion, insertion, addition, or inversion of the nucleotide includes mutation which naturally occurs, for example, based on the difference between individuals of a cucumber plant, the difference between varieties, the formation of multiple copies of the gene, the difference between respective organs, and the difference between respective tissues.

DNA having mutation as described above is expressed

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in an appropriate cell to investigate the raffinose  
synthase activity of an expressed product. Thus it is  
possible to obtain DNA which codes for substantially the  
same protein as the raffinose synthase. Further, DNA  
5 coding for substantially the same protein as the  
raffinose synthase protein can be obtained by isolating  
DNA which is hybridizable under a stringent condition  
with DNA having a nucleotide sequence comprising  
nucleotide residues having, for example, nucleotide  
10 numbers of 56 to 2407 in the nucleotide sequence shown  
in SEQ ID NO: 4 in Sequence Listing, and which codes for  
the protein having the raffinose synthase activity, from  
DNA's coding for raffinose synthases having mutation or  
from cells harboring the DNA's. The phrase "stringent  
15 condition" referred to herein indicates a condition in  
which so-called specific hybrid is formed, and  
nonspecific hybrid is not formed. It is difficult to  
definitely express this condition by using numerical  
values. However, for example, this condition includes a  
20 condition in which DNA's having high homology, for  
example, DNA's having homology of not less than 50 %  
hybridize with each other, while DNA's having homology  
lower than the above do not hybridize with each other,  
or a condition in which hybridization is achieved at a  
25 salt concentration corresponding to a washing condition  
for ordinary Southern hybridization, i.e., 1 x SSC, 0.1  
% SDS, and preferably 0.1 x SSC, 0.1 % SDS at 60 °C.

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Genes, which hybridize under such a condition, include those which contain a stop codon generated at an intermediate position, and those which have lost the activity due to mutation at the active center. However, those having such inconvenient mutation can be easily eliminated by ligating the gene with a commercially available activity expression vector to measure the raffinose synthase activity in accordance with the method described above.

- When the DNA of the present invention is used to express antisense RNA for the raffinose synthase, it is unnecessary for the DNA to code for any active raffinose synthase. Further, the function of any endogenous gene having homology can be restrained by using sense RNA.
- In such a case, it is also unnecessary for the DNA to code for any active raffinose synthase. Further, it is unnecessary for the DNA to contain the entire length. Preferably, it is sufficient for the DNA to have about 500 base pairs of an N-terminal side translating region having 60 % of homology.

The method has been explained above, in accordance with which the present inventors have succeeded in cloning the objective cDNA of the raffinose synthase originating from cucumber. However, other than the foregoing, the following methods may be available.

- (1) The raffinose synthase originating from cucumber is isolated and purified, and an entire

nucleotide sequence is chemically synthesized on the basis of a determined amino acid sequence or the amino acid sequence shown in SEQ ID NO: 5.

(2) Chromosomal DNA is prepared from a cucumber  
5 plant body, and a chromosomal DNA library is prepared by using a plasmid vector or the like. The raffinose synthase gene is obtained from the library by means of hybridization or PCR. It is assumed that the raffinose synthase gene originating from chromosome contains  
10 intron in its coding region. However, DNA divided into several parts by such intron is included in the DNA of the present invention provided that it codes for the raffinose synthase.

(3) Poly(A)<sup>+</sup>RNA is fractionated into fractions in  
15 accordance with the molecular weight or the like. The fractions are subjected to an in vitro translation system based on the use of wheat germ or rabbit reticulocyte to determine a fraction containing mRNA coding for a polypeptide having the raffinose synthase  
20 activity. An objective cDNA fragment is prepared and obtained from the fraction.

(4) An anti-cucumber raffinose synthase antibody is prepared. Elements of a cDNA library are incorporated into a protein expression vector, and an  
25 appropriate host is infected therewith to express proteins encoded by cDNA's. An objective cDNA may be screened by using the foregoing antibody.

(5) Appropriate primers are synthesized on the basis of amino acid sequences of peptide fragments, and a sequence containing the terminal is amplified by means of the RACE method, followed by cloning thereof.

5    <3> Method for producing raffinose of the present invention

In the method for producing raffinose of the present invention, the raffinose synthase is allowed to act on sucrose and galactinol to produce raffinose.

10    When the raffinose synthase is allowed to act on sucrose and galactinol, the galactose residue used for constructing galactinol is transferred to sucrose, and thus raffinose is produced. During this process, myo-inositol used for constructing galactinol is liberated.

15    The raffinose synthase, which is used to produce raffinose, may be an enzyme extracted from a plant body, or an enzyme produced by means of the genetic recombination technique based on the use of the DNA of the present invention.

20    In order to allow the raffinose synthase to act on sucrose and galactinol, the following procedure may be available. Namely, the raffinose synthase or cells having an ability to produce the raffinose synthase are immobilized to a carrier such as alginic acid gel and  
25    polyacrylamide gel to prepare immobilized enzyme or immobilized cells. The immobilized enzyme or the

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immobilized cells are charged to a column, and a solution containing sucrose and galactinol is allowed to pass through the column. As for the carrier and the method for immobilizing the raffinose synthase or the  
5 cells to the carrier, it is possible to adopt materials and methods which are used for ordinary bioreactors.

The raffinose synthesis reaction is performed, for example, by adding the raffinose synthase to a solution such as an aqueous solution or a buffer containing  
10 sucrose and galactinol. It is preferable that pH of the solution is adjusted to be within a range of about 6 to 8, especially at about pH 7. The reaction temperature is within a range of about 28 to 42 °C, preferably 35 to 40 °C, especially about 38 °C. The raffinose synthase  
15 of the present invention is stable within a range of pH 5 to 8, especially in the vicinity of pH 6. The enzyme of the present invention is stable within a temperature range of not more than about 40 °C.

The enzyme activity of the raffinose synthase of  
20 the present invention is inhibited by iodoacetamide, N-ethylmaleimide,  $MnCl_2$ ,  $ZnCl_2$ , and  $NiCl_2$ . Therefore, it is desirable that these substances are not contained in the reaction solution.

Preferably, galactinol and sucrose are added to the  
25 reaction solution at a concentration of not less than 5 mM of galactinol and a concentration of not less than 1.5 mM of sucrose. The raffinose synthase may be added

to the reaction solution in an amount depending on the amounts of the substrates.

Raffinose is separated from unreacted sucrose and galactinol and from myo-inositol produced by the enzyme reaction, contained in the reaction solution, in accordance with a method including, for example, gel filtration chromatography.

<4> Chimeric gene and transgenic plant of the present invention

10       The chimeric gene of the present invention includes the raffinose synthase gene or a part thereof and the transcription regulatory region expressible in plant cells. The raffinose synthase gene is exemplified by the DNA coding for the raffinose synthase of the present invention described in the foregoing item <2>. When the  
15       chimeric gene of the present invention is used as an antisense gene, a non-coding region of the raffinose synthase gene or a part thereof can be used in some cases, besides the DNA coding for the raffinose  
20       synthase. The non-coding region includes, for example, sequences indicated by nucleotide numbers of 1 to 55 (5'-non-coding region) and nucleotide numbers of 2407 to 2517 (3'-non-coding region) in SEQ ID NO: 4 in Sequence Listing.

25       When the transcription regulatory region is linked to the DNA coding for the raffinose synthase in the

chimeric gene of the present invention so that mRNA  
(sense RNA) homologous to the coding strand of the DNA  
is expressed, plant cells introduced with the chimeric  
gene express the raffinose synthase, and the content of  
5 the raffinose family oligosaccharides is increased. On  
the other hand, when the transcriptional regulatory  
region is linked to the DNA so that RNA (antisense RNA)  
having a sequence complementary to the coding strand of  
the DNA is expressed, and when the transcription  
10 regulatory region is linked to the DNA so that a partial  
fragment of the raffinose synthase gene, preferably  
sense RNA for a portion of not less than about 200 base  
pairs in the upstream coding region is expressed, then  
the expression of endogenous raffinose synthase is  
15 restrained in plant cells introduced with the chimeric  
gene, and the raffinose family oligosaccharides are  
decreased.

The content of the raffinose family  
oligosaccharides in a plant can be changed by  
20 transforming the plant with the chimeric gene of the  
present invention, and expressing the gene in cells of  
the plant.

Plants to which the present invention is applicable  
include, for example, oil crops such as soybean,  
25 rapeseed, cotton; sugar crops such as sugar beet and  
sugar cane; and model plants represented by Arabidopsis  
thaliana.

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The transcription regulatory region expressible in plant cells includes, for example, promoters which make expression over a whole plant, such as CaMV 35S RNA promoter, CaMV 19S RNA promoter, and nopaline synthase promoter; promoters which make expression in green tissues, such as Rubisco small subunit promoter; and promoter regions which make site-specific expression at portions such as seed, including, for example, those for genes of napin and phaseolin as described above. The 3'-terminal of the chimeric gene may be connected with the terminator such as nopaline synthase terminator, and Rubisco small subunit 3'-end portion.

The plant may be transformed with the chimeric gene in accordance with usually used methods such as the Agrobacterium method, the particle gun method, the electroporation method, and the PEG method, depending on the host cell to be manipulated.

The transformation method for introducing the chimeric gene into the plant includes, for example, the Agrobacterium method, the particle gun method, the electroporation method, and the PEG method.

The Agrobacterium method is specifically exemplified by a method based on the use of a binary vector. Namely, a plant is infected with a vector comprising T-DNA originating from Ti plasmid, a replication origin which is functional in microorganisms such as Escherichia coli, and a marker gene for

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selecting plant cells or microbial cells harboring the vector. Seeds are collected from the plant, and they are allowed to grow. Plants introduced with the vector are selected by using an index of expression of the marker gene. Obtained plants are measured for the raffinose synthase activity, or strains exhibiting change in content of the raffinose family oligosaccharides are selected from the obtained plants. Thus it is possible to obtain an objective transformed plant.

A method for introducing the chimeric gene into soybean will be described below. In order to perform transformation for soybean, it is possible to use any one of the particle gun method (Pro. Natl. Acad. Sci. USA, 86, 145 (1989); TIBTECH, 8, 145 (1990); Bio/Technology, 6, 923 (1988); Plant Physiol, 87, 671 (1988); Develop. Genetics, 11, 289 (1990); and Plant cell Tissue & Organ Culture, 33, 227 (1993)), the Agrobacterium method (Plant Physiol., 91, 1212 (1989); WO 94/02620; Plant Mol. Biol., 9, 135 (1987); and Bio/Technology, 6, 915 (1988)), and the electroporation method (Plant Physiol, 99, 81 (1992); Plant Physiol, 84, 856 (1989); and Plant Cell Reports, 10, 97 (1991)).

In the particle gun method, it is preferable to use an embryogenic tissue or a hypocotyl of an immature seed about 30 to 40 days after dehiscence of anthesis. About 1 g of the embryogenic tissue is spread over a petri

dish, into which, for example, gold particles or tungsten particles coated with the objective chimeric gene may be shot. The tissue is transferred after 1 to 2 hours to a liquid medium to perform cultivation.

- 5 After 2 weeks, the tissue is transferred to a medium containing an antibiotic for transformant selection, followed by cultivation. After 6 weeks, a green adventitious embryo which is resistant to the antibiotic is obtained. The adventive embryo is further
- 10 transferred to a fresh medium and cultured so that a plant body is reproduced. Alternatively, when the hypocotyl is used, the hypocotyl is excised under a sterilized condition, and it is treated in accordance with the particle gun method, followed by cultivation in
- 15 MS medium (Murashige and Skoog, Physiologia Plantarum, 15, 473-497 (1962)) containing cytokinin at a high concentration. The hypocotyl is cultured in the darkness for 2 weeks, and then it is cultured at room temperature with light irradiation for 12 to 16 hours in
- 20 MS medium having a lowered cytokinin content. During this process, it is preferable to add, to the medium, the antibiotic having been used as the selection marker. When a multiple bud body is formed from the transplanted tissue, it is transferred to a medium added with no
- 25 hormone so that rooting is caused. An obtained seedling body is transferred to a greenhouse and cultivated.

In the case of the method based on the use of

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Agrobacterium, it is desirable to use Cotyledonary nod as a plant tissue. Commercially available LBA4404, C58, and Z707 can be used as Agrobacterium. However, it is desirable to use Z707. For example, a plasmid obtained by inserting the objective gene into pMON530 (produced by Monsanto Co.) can be used as the vector. The plasmid is introduced into Agrobacterium tumefaciens Z707 (Hepburn et al., J. Gen. Microbiol., 131, 2961 (1985)) in accordance with, for example the Direct freeze thaw method (An et al., "Plant Mol. Biol. Manual", A3: 1-19, 1988). The Agrobacterium transformed with the chimeric gene is cultivated overnight. Proliferated cells are collected by centrifugation at 5000 rpm for 5 minutes, and they are suspended in B5 suspension medium. Soybean seeds are sterilized, and they are cultivated for 3 days on B5 medium having a 1/10 concentration so that they germinate. Cotyledons are excised, and they are cultivated for 2 hours with the suspension of Agrobacterium. The cotyledons are transferred to B5 medium (containing Gamborg B5 salt (Exp. Cell. Res., 50, 151 (1968)), Gamborg B5 vitamin, 3 % sucrose, 5  $\mu$ M benzylaminopurine, 10  $\mu$ M IBA, and 100  $\mu$ M acetosyringon), and they are cultivated for 3 days under a condition at 25 °C with light irradiation ( $60 \mu\text{Em}^{-2}\text{S}^{-1}$ ) for 23 hours. Subsequently, in order to remove Agrobacterium, the cotyledons are cultivated in B5 medium (5  $\mu$ M benzylaminopurine, 10 mg/L carbenicillin, 100 mg/L

vancomycin, and 500 mg/L cefotaxime) at 25 °C for 4 days while exchanging the medium every day. After that, the cotyledons are cultivated in B5 medium (200 mg/L kanamycin). Multishoots are formed within 1 or 2 months. They are cultivated on B5 medium (0.58 mg/L gibberellin and 50 mg/L kanamycin) to elongate the shoots. Subsequently, the shoots are transferred to B5 medium (10 µM IBA) to cause rooting. Rooted seedlings are acclimatized, and they are cultivated in a greenhouse. Thus transformants can be obtained.

A transformant plant, in which the raffinose synthase gene is introduced, can be easily confirmed by extracting DNA from the transformant, and performing Southern hybridization by using the raffinose synthase gene as a probe.

#### Brief Description of the Drawings

Fig. 1 shows a relationship between the reaction time and the amount of raffinose produced by the raffinose synthesis reaction.

Fig. 2 shows a photograph illustrating a result of SDS-polyacrylamide gel electrophoresis for the raffinose synthase. M indicates molecular weight markers, and S indicates a sample containing the raffinose synthase. Numerals indicate molecular weights (kDa).

Fig. 3 shows an influence of the reaction

temperature on the raffinose synthase activity.

Fig. 4 shows an influence of the reaction pH on the raffinose synthase activity.

Fig. 5 shows an influence of myo-inositol on the raffinose synthase activity.

Fig. 6 shows a stable pH range of the raffinose synthase.

Fig. 7 shows relationships between synthetic primers and amino acid sequences of peptides. R represents A or G, Y represents C or T, M represents A or C, K represents G or T, D represents G, A, or T, H represents A, T, or C, B represents G, T, or C, N represents G, A, T, or C, and I represents inosine.

#### Best Mode for Carrying Out the Invention

The present invention will be more specifically explained below with reference to Examples.

At first, the method for measuring the raffinose synthase activity, used to confirm active fractions during respective purification steps and investigate characteristics of the enzyme in the following Examples, will be explained.

#### <Method for measuring the raffinose synthase activity>

The activity of the raffinose synthase was measured by quantitatively determining raffinose produced by the

raffinose synthesis reaction by using HPLC (high-performance liquid chromatography). HPLC was performed by using Sugar Analysis System DX500 (CarboPac PA1 column, pulsed amperometry detector (produced by  
5 DIONEX)).

The raffinose synthesis reaction was performed by using a reaction solution prepared to have a composition having the following final concentrations. The reaction solution was added with 10 to 50  $\mu$ l of a raffinose  
10 synthase solution to give a volume of 100  $\mu$ l, followed by performing the reaction at 32 °C for 60 minutes.

[Composition of reaction solution (final concentration)]

2.5 mM sucrose  
5 mM galactinol  
15 5 mM DTT  
20 mM Tris-HCl buffer (pH 7.0)

After performing the reaction as described above, the reaction solution was added with ethanol in a volume four times the volume of the reaction solution to stop  
20 the reaction by heating the solution at 95 °C for 30 seconds. The obtained solution was centrifuged to obtain a supernatant which was then dried up under a reduced pressure. After that, an obtained residue was dissolved in distilled water. Raffinose in the reaction  
25 product was quantitatively determined by using the sugar

analysis system to estimate the raffinose synthase activity.

Example 1: Purification of Raffinose Synthase  
from Cucumber

5    <1> Extraction of raffinose synthase from cucumber

Vein tissues were collected from true leaves of cucumber (cv.: SUYOU) obtained 6 to 10 weeks after planting. The leaf vein tissues were frozen with liquid nitrogen, and they were stored at -80 °C. The frozen  
10    leaf vein tissues were ground by a mortar with liquid nitrogen, to which Buffer 1 (40 mM Tris-HCl buffer (pH 7.0), 5 mM DTT, 1 mM PMSF (phenylmethanesulfonyl fluoride), 1 % polyclarl AT (produced by Serva)) was added to extract proteins. An obtained extract solution  
15    was filtrated with a filter such as gauze or Miracloth (produced by Calbiochem-Novobiochem). An obtained filtrate was centrifuged at 4 °C at about 30,000 x g for 60 minutes. A supernatant obtained by the centrifugation was used as a crude extract solution.

20    <2> Anion exchange chromatography (1)

The crude extract solution (about 560 ml) obtained as described above was applied to a column system comprising five connected columns for strongly basic anion exchange chromatography (HiTrap Q, produced by

Pharmacia, 1.6 cm x 2.5 cm) equilibrated with Buffer 2 (20 mM Tris-HCl buffer (pH 7.0), 5 mM DTT) to adsorb the raffinose synthase activity to the columns.

Subsequently, the columns were washed with Buffer 3 (20 mM Tris-HCl buffer (pH 7.0), 0.2 M NaCl, 5 mM DTT) in a volume five times of the columns so that non-adsorbed proteins were washed out. After that, the raffinose synthase activity was eluted from the columns with 50 ml of Buffer 4 (20 mM Tris-HCl buffer (pH 7.0), 0.3 M NaCl, 5 mM DTT).

### <3> Anion exchange chromatography (2)

The eluted solution (about 75 ml) was placed in a dialysis tube (Pormembranes MWC 0:10,000, produced by Spectra), and it was dialyzed against 10 L of Buffer 5 (20 mM Tris-HCl buffer (pH 7.0), 0.05 M NaCl, 5 mM DTT) at 4 °C overnight. The dialyzed sample was applied to a column for weakly basic anion exchange chromatography (DEAE-TOYOPEARL, produced by Toyo Soda, 2.2 x 20 cm) equilibrated with Buffer 5 to adsorb the raffinose synthase activity to the column. Subsequently, the column was washed with Buffer 5 in a volume five times the volume of the column to wash out non-adsorbed proteins. After that, a linear concentration gradient of 0.05 M to 0.35 M NaCl in a volume twenty times the volume of the column was applied to elute the enzyme activity so that fractionation was performed.

<4> Gel filtration chromatography

The eluted solution obtained as described above (about 160 ml) was concentrated into 6.5 ml by using a concentrator (Centriprep 10, produced by Amicon).

5 Aliquots (each 3 ml) of the concentrated solution were applied to a column for gel filtration chromatography (Superdex 200 pg, produced by Pharmacia, 2.6 cm x 60 cm). Equilibration for the column and elution from the  
10 column were performed by using Buffer 6 (20 mM Tris-HCl buffer (pH 7.0), 0.1 M NaCl, 5 mM DTT, 0.02 % Tween 20). Fractions having the raffinose synthase activity were collected from fractionated fractions.

<5> Hydroxyapatite chromatography

A collected fraction (about 25 ml) having the  
15 raffinose synthase activity fractionated by the gel filtration was concentrated by using Centriprep 10, and the buffer was exchanged with Buffer 7 (0.01 M sodium phosphate buffer (pH 7.0), 5 mM DTT, 0.02 % Tween 20). An obtained concentrate solution (about 1.2 ml) was  
20 applied to a hydroxyapatite column (Bio-Scale CHT-1, produced by Bio Rad, 0.7 x 5.2) previously equilibrated with the same buffer to adsorb the raffinose synthase activity. The column was washed with the same buffer in a volume (10 ml) five times the volume of the column.  
25 After that, a linear concentration gradient of 0.01 M to 0.3 M phosphate in a volume twenty times the volume of

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the column was applied to elute the enzyme activity so that fractionation was performed.

<6> Hydroxyapatite rechromatography

An active fraction obtained in accordance with the hydroxyapatite chromatography as described above was subjected to rechromatography in the same manner as described above to obtain a purified raffinose synthase fraction (about 2 ml).

The amount of protein contained in the active fraction was about 200 µg. The total activity was 5700 nmol/hour, and the specific activity per protein was 28 µmol/hour/mg. The active fraction contained only a protein which exhibited a single band corresponding to a molecular weight of 90 kDa to 100 kDa on electrophoresis as described later on. The specific activity of the obtained purified enzyme sample was about 2000 times that of the crude extract solution. The recovery was 12 % with respect to the amount of the enzyme obtained after the strongly basic anion exchange chromatography based on the use of HiTrap Q. Results of the purification are summarized in Table 1.

Table 1

		<u>Total</u>	<u>Total</u>	<u>Specific</u>	<u>Yield</u>
		<u>protein</u>	<u>activity</u>	<u>activity</u>	<u>%</u>
		<u>mg</u>	<u>nmol/h</u>	<u>nmol/h/mg</u>	
5	Crude extract	1915	20700	11	-
	HiTrap Q	1092	48800	45	100
	DEAE-TOYOPEARL	540	33000	61	68
	Superdex 200 pg	1.79	26500	14800	54
	Apatite (1)*	0.51	12600	24700	26
10	Apatite (2)*	0.20	5700	28500	12

Apatite (1)\*: Hydroxyapatite chromatography (1)

Apatite (2)\*: Hydroxyapatite chromatography (2)

Example 2: Investigation on Characteristics  
of Raffinose Synthase

15            Characteristics of the purified raffinose synthase  
obtained in Example 1 were investigated.

<1> Molecular weight measurement

(1) Gel filtration chromatography

20            An aliquot (10 µl) of the purified raffinose  
synthase was dispensed. This sample and a molecular  
weight marker (Molecular Weight Marker Kit for Gel  
Filtration, produced by Pharmacia) were applied to a gel

filtration chromatography column (Superdex 200 pg, produced by Pharmacia). Equilibration of the column and elution from the column were performed by using Buffer 6 (20 mM Tris-HCl buffer (pH 7.0), 0.1 M NaCl, 5 mM DTT, 0.02 % Tween 20). As a result, the molecular weight of the raffinose synthase was estimated to be about 75 kDa to 95 kDa.

(2) Polyacrylamide gel electrophoresis (Native PAGE)

An aliquot (10 µl) of the purified raffinose synthase was dispensed, to which the same volume of a sample buffer (0.0625 M Tris-HCl (pH 6.8), 15 % glycerol, 0.001 % BPB) was added to prepare an electrophoresis sample. The sample (10 µl) was applied to 10 % polyacrylamide gel (produced by Daiichi Chemical, Multigel 10), and electrophoresed at 40 mA for about 60 minutes with 0.025 M Tris - 0.192 M glycine buffer (pH 8.4). After the electrophoresis, the gel was stained with Silver Stain Kit (produced by nacalai tesque). As a result, the molecular weight was estimated to be about 90 kDa to 100 kDa.

(3) SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

An aliquot (10 µl) of the purified raffinose synthase was dispensed, to which the same volume of a sample buffer (0.0625 M Tris-HCl (pH 6.8), 2 % SDS, 10 % glycerol, 5 % mercaptoethanol, 0.001 % BPB) was added, followed by heating in a boiling water bath for 1 minute to prepare an electrophoresis sample. The sample (10

005250" 8029/960  
5  $\mu$ l) was applied to 10 to 20 % gradient polyacrylamide gel (produced by Daiichi Chemical), and electrophoresed at 40 mA for about 70 minutes with 0.025 M Tris - 0.192 M glycine buffer (pH 8.4) containing 0.1 % SDS. After the electrophoresis, the gel was stained with Silver Stain Kit (produced by nacalai tesque). A result is shown in Fig. 2. As a result, the molecular weight was estimated to be about 90 kDa to 100 kDa.

#### <2> Optimum reaction temperature

10 The raffinose synthase activity was measured under various temperature conditions (28 °C, 32 °C, 36 °C, 40 °C, 44 °C, 48 °C, and 52 °C) in accordance with the method for measuring the raffinose synthase activity described above. The enzyme solution was added to the  
15 respective reaction solutions in an amount of 2  $\mu$ l. Fig. 3 shows relative activities at the respective temperatures assuming that the enzyme activity at 32 °C was 100. As a result, the raffinose synthase exhibited the activity in a range of about 25 to 42 °C, and the  
20 optimum reaction temperature was about 35 to 40 °C.

#### <3> Optimum reaction pH

The raffinose synthase activity was measured under various pH conditions (pH 4 to 11) in accordance with the method for measuring the raffinose synthase activity  
25 described above. The respective reactions were

performed by using 50 mM citrate buffer (pH 4 to 6), 50 mM potassium phosphate buffer (pH 5.5 to 7.5), 50 mM Bis-Tris buffer (pH 6 to 7), 20 mM Tris-HCl buffer (pH 7 to 8.5), and 50 mM glycine-NaOH buffer (pH 9 to 11).

5 The enzyme solution was added to the respective reaction solutions in an amount of 2  $\mu$ l. A result is shown in Fig. 4.

As a result, the raffinose synthase exhibited the activity in a range of pH 5 to 10, and the optimum  
10 reaction pH was about 6 to 8, provided that the activity varied depending on the type of the buffer used for the measurement.

#### <4> Investigation on inhibitors and metal ions

Various enzyme inhibitors or metal ions were added  
15 to the reaction solution of the purified raffinose synthase to give a final concentration of 1 mM respectively, and the raffinose synthase activity was measured in the same manner as described above. Table 2 shows remaining activities with respect to the enzyme  
20 activity obtained when neither inhibitor nor metal ion was added. Iodoacetamide and N-ethylmaleimide clearly inhibited the enzyme activity. The inhibiting effect was scarcely observed for  $\text{CaCl}_2$ ,  $\text{CuCl}_2$ , and  $\text{MgCl}_2$ . However,  $\text{MnCl}_2$ ,  $\text{ZnCl}_2$ , and  $\text{NiCl}_2$  exhibited the inhibiting  
25 effect.

Table 2

	<u>Inhibitor or metal ion</u>	<u>Remaining activity (%)</u>
	No addition	100
	Iodoacetoamide	0
5	N-ethylmaleimide	40
	CaCl <sub>2</sub>	115
	CuCl <sub>2</sub>	101
	MgCl <sub>2</sub>	96
	MnCl <sub>2</sub>	32
10	ZnCl <sub>2</sub>	42
	NiCl <sub>2</sub>	68

<5> Inhibition by myo-inositol

Investigation was made for inhibition by myo-inositol as the reaction product of the raffinose synthesis reaction. The reaction solution was added with myo-inositol at various concentrations, and the raffinose synthase activity was measured. A result is shown in Fig. 5. The enzyme activity was inhibited as the concentration of added myo-inositol was increased.

20 <6> Stable pH

The raffinose synthase fraction obtained by the anion exchange chromatography (2) described above was incubated for 4 hours at 4 °C in 50 mM Bis-Tris-HCl buffer (pH 5 to 8.0, containing 0.5 mM DTT) or 20 mM

006260" 8025/960

Tris-HCl buffer (pH 7 to 8.0, containing 0.5 mM DTT),  
and then the raffinose synthase activity was measured.  
Fig. 6 shows the enzyme activity versus pH of the buffer  
used for the incubation. The raffinose synthase  
5 activity was confirmed after the incubation under any of  
the incubation conditions. Especially, the enzyme was  
stable in a range of pH 5 to 7.5.

#### <7> Stable temperature

The raffinose synthase fraction obtained by the  
10 anion exchange chromatography (2) described above was  
incubated in 20 mM Tris-HCl buffer (pH 7, containing 0.5  
mM DTT) for 60 minutes at 28 °C, 32 °C, 37 °C, or 40 °C,  
and then the raffinose synthase activity was measured.  
As a result, the enzyme of the present invention was  
15 stable, exhibiting, in the range of 28 °C to 40 °C,  
activities of 80 % to 100 % of that obtained by a  
control for which the incubation treatment was not  
performed for comparison.

#### <8> Analysis of amino acid sequence

20 The cysteine residue of the purified raffinose  
synthase was subjected to reducing pyridylethylation,  
and the reaction mixture was desalted. An obtained  
sample was digested at 37 °C for 12 hours with  
lysylendopeptidase (Achromobacter protease 1, produced  
25 by Wako Pure Chemical Industries) to form peptide

fragments. An obtained peptide mixture was applied to reverse phase HPLC (column: Waters  $\mu$ Bondasphere ( $\phi$ 2.1 x 150 mm, C<sub>18</sub>, 300 Å, produced by Waters (Millipore))) to separate and obtain the respective peptide fragments.

5 0.1 % TFA (trifluoroacetic acid) was used as a solvent, and elution was performed with a concentration gradient of acetonitrile. Amino acid sequences of three fragments selected from the obtained peptide fragments were determined by using a protein sequencer. The  
10 determined amino acid sequences of the respective peptides are shown in SEQ ID NOs: 1 to 3 in Sequence Listing. These peptides will be thereafter referred to as Peptides 1, 2, and 3 respectively in this order.

### Example 3: Preparation of DNA

#### 15 Coding for Raffinose Synthase

#### <1> Isolation of partial fragment of cDNA of raffinose synthase by means of PCR method

Major veins of cucumber (22 g) were ground by a mortar with liquid nitrogen. The ground material was  
20 added to a mixture of an extraction buffer (100 mM lithium chloride, 100 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 1 % SDS) and an equal amount of phenol previously heated to 80 °C, followed by agitation. After that, a mixture of phenol and an equal amount of chloroform:  
25 isoamyl alcohol (24:1) was added thereto, followed by

agitation again. An obtained mixture solution was centrifuged at 4 °C at 9250 x g for 15 minutes to collect a supernatant. The supernatant was repeatedly subjected to the treatment with phenol and the treatment  
5 with chloroform: isoamyl alcohol to obtain a supernatant after centrifugation. The supernatant was added with an equal amount of 4 M lithium chloride, followed by being stationarily left to stand at -70 °C for 1 hour.

After thawing at room temperature, the sample was  
10 treated and centrifuged at 4 °C at 9250 x g for 30 minutes to obtain a precipitate. The precipitate was washed with 2 M lithium chloride once and with 80 % ethanol once. After drying, the precipitate was dissolved in 2 ml of a diethylpyrocarbonate-treated  
15 solution to give a sample of purified total RNA. The obtained total RNA was 2.38 mg.

The all amount of the total RNA was applied to poly(A)<sup>+</sup>RNA purification kit (produced by STRATAGENE CLONING SYSTEMS) based on the use of an oligo(dT)  
20 cellulose column so that poly(A)<sup>+</sup>RNA molecules were purified to obtain 42.5 µg of poly(A)<sup>+</sup>RNA.

Single strand cDNA's were synthesized from poly(A)<sup>+</sup>RNA obtained as described above, by using reverse transcriptase Super Script II (produced by GIBCO  
25 BRL). In order to isolate raffinose synthase cDNA from an obtained cDNA mixture, amplification was performed in accordance with the PCR method. In order to be used as

primers in PCR, single strand oligonucleotides (SEQ ID  
NOs: 6 to 22) shown in Fig. 7 were synthesized on the  
basis of the amino acid sequences of the peptide  
fragments of the raffinose synthase originating from  
5 cucumber, determined in Example 2. In the sequences of  
the respective primers, R represents A or G, Y  
represents C or T, M represents A or C, K represents G  
or T, D represents G, A, or T, H represents A, T, or C,  
B represents G, T, or C, N represents G, A, T, or C, and  
10 I represents inosine (base: hypoxanthine) respectively.

A DNA fragment of about 540 base pairs was  
amplified when the primers were combined and used such  
that the 5'-side primer was A (A1 (SEQ ID NO: 6), A2  
(SEQ ID NO: 7), A3 (SEQ ID NO: 8), A4 (SEQ ID NO: 9))  
15 and the 3'-side primer was D' (D'1 (SEQ ID NO: 21), D'2  
(SEQ ID NO: 22)), or the 5'-side primer was C2 (SEQ ID  
NO: 14) and the 3'-side primer was B'1 (SEQ ID NO: 18)  
or B'2 (SEQ ID NO: 19). The fragment was cloned into a  
plasmid pCRII by using TA cloning kit (produced by  
20 INVITROGEN BV) to analyze its nucleotide sequence. As a  
result, a nucleotide sequence coding for the amino acid  
sequences of Peptides 1, 2 was found inwardly between  
the primer sequences at both terminals. Accordingly, it  
was found that the amplified fragment is a DNA fragment  
25 originating from the raffinose synthase gene.

In order to specify the position of the cloned PCR-  
amplified DNA fragment on the raffinose synthase gene,

006260" 8029/960

3'-RACE was performed by using RACE kit (3' Ampifinder RACE Kit, produced by CLONTACH).

5 PCR was performed by using the cDNA mixture as a template, C (C1 (SEQ ID NO: 13), C2 (SEQ ID NO: 14)) as a 5'-side primer, and a primer having oligo(dT) and an anchor sequence as a 3'-side primer. Further, PCR was performed by using an amplified fragment thus obtained as a template, D (D1 (SEQ ID NO: 15), D2 (SEQ ID NO: 16)) located inwardly from C as a 5'-side primer, and an  
10 oligo(dT)-anchor primer as a 3'-side primer. As a result, a DNA fragment of about 2400 base pairs was amplified only when PCR was performed by using, as the template, DNA amplified with C1 (SEQ ID NO: 13) or C2 (SEQ ID NO: 14) and the oligo(dT)-anchor primer, and  
15 using D2 (SEQ ID NO: 16) and the oligo(dT)-anchor primer. Further, PCR was performed by using C (C1 (SEQ ID NO: 13), C2 (SEQ ID NO: 14)) as the 5'-side primer and the oligo(dT)-anchor primer as the 3'-side primer, and then PCR was performed by using the amplified  
20 fragment thus obtained as a template, E (SEQ ID NO: 17) as a 5'-side primer, and the oligo(dT)-anchor primer as a 3'-side primer. As a result, a DNA fragment of about 300 base pairs was amplified in any case.

25 Similarly, PCR was performed by using A (A1 (SEQ ID NO: 6), A2 (SEQ ID NO: 7), A3 (SEQ ID NO: 8), or A4 (SEQ ID NO: 9)) as a 5'-side primer, and the primer having oligo(dT) and the anchor sequence as a 3'-side primer.

Further, PCR was performed by using an amplified  
fragment thus obtained as a template, and using B (B1  
(SEQ ID NO: 10), B2 (SEQ ID NO: 11), or B3 (SEQ ID NO:  
12)) located inwardly from A as a 5'-side primer, and  
5 the same oligo(dT)-anchor primer as a 3'-side primer.  
As a result, a DNA fragment of about 2000 base pairs was  
obtained when the B2 primer was used even when any of  
the A primers was used. Thus the DNA fragment amplified  
by using the A2 and B2 primers was cloned. As a result  
10 of nucleotide sequence analysis, the DNA fragment  
included the nucleotide sequence coding for the amino  
acid sequence of Peptide fragment 1 used to prepare the  
5'-side primer. The DNA fragment also included, on the  
3'-side, the poly(A) sequence and the nucleotide  
15 sequence corresponding to Peptide fragment 3 at a  
position located upstream therefrom.

In view of the result of PCR described above, it  
was found that Peptide fragments of the raffinose  
synthase are arranged from the N-terminal side in an  
20 order of 2, 1, 3, and the DNA fragment of about 540 base  
pairs previously obtained by PCR was a portion located  
near to the 5'-terminal on the raffinose synthase gene.  
In order to screen a cDNA clone containing the entire  
length of the raffinose synthase gene, it is desirable  
25 that DNA to be used as a probe can detect a portion near  
to the 5'-terminal side. Accordingly, the obtained DNA  
fragment was used as a probe to perform screening for a

cDNA library.

<2> Cloning of entire length of coding region of  
raffinose synthase cDNA

At first, a cDNA library was prepared as follows.

5 Double strand cDNA's were synthesized from poly(A)<sup>+</sup>RNA  
(3.8 µg) obtained in the foregoing item <1> by using  
Time Saver cDNA synthesis kit (produced by Pharmacia  
Biotech). Obtained cDNA's were incorporated into EcoRI  
restriction enzyme cleavage site of λ phage vector,  
10 λMOSSlox (produced by Amersham) respectively, which were  
then incorporated into the phage protein by using  
GigapackII Gold packaging kit (produced by STRATAGENE  
CLONING SYSTEMS). Thus the cucumber cDNA library was  
prepared. This library had a titer of  $1.46 \times 10^7$  pfu/µg  
15 vector.

Host cells of Escherichia coli ER1647 were infected  
with the phages contained in the cucumber cDNA library  
in an amount corresponding to  $1.4 \times 10^5$  pfu, and then  
the cells were spread over 14 agar plates each having a  
20 diameter of 90 mm to give  $1.0 \times 10^4$  pfu per one plate.  
The cells were cultivated at 37 °C for about 6.5 hours.  
After that, phage plaques formed on the plates were  
transferred to nylon membranes (Hybond-N+, produced by  
Amersham).

25 Next, the nylon membranes were treated with alkali  
to denature transferred DNA, followed by neutralization

and washing. After that, the nylon membranes were treated at 80 °C for 2 hours to fix DNA on the membranes.

Positive clones were screened on the obtained nylon  
5 membrane by using the DNA fragment of about 540 base  
pairs obtained in the foregoing item <1> as a probe.  
The DNA fragment of about 540 base pairs was digested  
with restriction enzyme EcoRI, followed by  
electrophoresis to excise and purify only the insert of  
10 about 540 base pairs. The insert was labeled with  
fluorescein by using DNA labeling and detection system  
(Gene Images labeling and detection system, produced by  
Amersham) to be used as the probe. The nylon membranes  
were subjected to prehybridization at 60 °C for 30  
15 minutes, and then the labeled probe was added to perform  
hybridization at 60 °C for 16 hours. An antibody  
(alkaline phosphatase-labeled anti-fluorescein antibody)  
for detecting the labeled DNA was used after being  
diluted 50000 times. In this screening process,  
20 candidate strains for positive clones were obtained.  
The obtained candidate strains were further subjected to  
repeated screening twice in the same manner as described  
above to obtain a purified positive clone.

Escherichia coli BM25.8 was infected with the  
25 positive clone, and it was cultivated on a selection  
medium containing carbenicillin. A plasmid vector  
λMOSSlox-CRS containing cDNA was excised therefrom. The

inserted cDNA of the plasmid had a length of about 2.5 kb. Escherichia coli JM109 was transformed with the plasmid. Plasmid DNA was prepared from a transformant, which was used as a sample for analyzing the nucleotide  
5 sequence.

The nucleotide sequence of the inserted cDNA was analyzed by using Taq DyeDeoxy Terminator Cycle Sequencing Kit (produced by Perkin-Elmer) in accordance with the conventionally known method.

10 As a result, a nucleotide sequence comprising 2352 base pairs as shown in SEQ ID NO: 4 in Sequence Listing was revealed. The sequence included a portion coincident with the nucleotide sequence of the DNA probe used by the present inventors. An amino acid sequence  
15 translated from the nucleotide sequence is shown in SEQ ID NOs: 4 and 5. The amino acid sequence included portions coincident with Peptide 1 (amino acid numbers of 215 to 244 in SEQ ID NO: 5), Peptide 2 (amino acid numbers of 61 to 79 in SEQ ID NO: 5), and Peptide 3  
20 (amino acid numbers of 756 to 769 in SEQ ID NO: 5) of the raffinose synthase originating from cucumber obtained by the present inventors. Thus it was confirmed that the amino acid sequence codes for the raffinose synthase.

25 The transformant, designated as AJ13263, of Escherichia coli JM109, which harbors the plasmid pMossloxCRS containing DNA coding for the raffinose

synthase obtained as described above, has been  
internationally deposited on the basis of the Budapest  
Treaty since November 19, 1996 in National Institute of  
Bioscience and Human Technology of Agency of Industrial  
5 Science and Technology of Ministry of International  
Trade and Industry (postal code: 305, 1-3 Higashi-  
Icchome, Tsukuba-shi, Ibaraki-ken, Japan), and awarded a  
deposition number of FERM BP-5748.

10       Example 4: Chimeric gene and Transformed Plant  
          Containing DNA Coding for Raffinose Synthase

<1> Construction of plasmid containing chimeric gene

          The DNA fragment coding for the raffinose synthase  
was introduced into Arabidopsis thaliana by using  
LBA4404 as Agrobacterium and pBI121 (produced by  
15 CLONTECH) as a binary vector. pBI121 is a plasmid  
originating from pBIN19, which comprises nopaline  
synthase gene promoter connected to neomycin  
phosphotransferase structural gene (NPTII), nopaline  
synthase gene terminator (Nos-ter), CaMV 35S promoter,  
20 GUS ( $\beta$ -glucuronidase) gene, and Nos-ter, and which has  
sequences for enabling transposition to plant, on both  
sides thereof. A SmaI site is located downstream from  
CaMV 35S promoter. An insert inserted into this site is  
expressed under the regulation of the promoter.

25       A fragment of the raffinose synthase gene obtained

in Example 3 was inserted into the binary vector pBI121. The raffinose synthase gene was digested with DraI to prepare, by means of agarose gel electrophoresis, a DNA fragment containing 30th to 1342th nucleotides in SEQ ID NO: 4 in Sequence Listing. This fragment was ligated into the SmaI site of pBI121. Escherichia coli HB101 was transformed with the ligation reaction solution to obtain transformant strains from which recombinant plasmids were prepared. Two recombinant plasmids, in which the raffinose synthase DNA fragment was reversely connected to CaMV 35S promoter (antisense), and the raffinose synthase DNA fragment was connected to CaMV 35S promoter in the positive direction (sense), were selected from the obtained recombinant plasmids. The two recombinant plasmids were designated as pBIRS1 and pBIRS9 respectively.

Each of the plasmids obtained as described above was introduced into Agrobacterium LBA4404 by means of triparental mating.

Arabidopsis thaliana was transformed as follows. Seeds of Arabidopsis thaliana was subjected to a treatment for water absorption. After that, they were sterilized by treating them with 80 % ethanol containing 1 % Tween 20 for 5 minutes, and treating them with 10 % sodium hypochlorite solution also containing 1 % Tween 20 for 10 minutes, followed by washing five times with sterilized water. The seeds were suspended in 1 % low

melting point agarose, and they were spread over an MS medium (MS basic medium (Murashige and Skoog, Physiologia Plantarum, 15, 473-497 (1962)), B5 vitamin, 10 g/L sucrose, 0.5 g/L MES, pH 5.8). The seeds were

5 cultivated at 22 °C for 1 week in a culture room to give a cycle comprising light irradiation for 16 hours and darkness for 8 hours. Plants with unfolded seed leaves were subjected to setting with rock wool. Cultivation was continued under the same condition. After about 3

10 weeks, decapitation was performed when the plants caused bolting to have heights of stems of several cm's. The plants were allowed to grow until a state in which first flowers bloom on elongated branches 1 week after the decapitation.

15 Agrobacterium harboring the introduced recombinant plasmid containing the raffinose synthase gene was precultivated in 2 ml of LB medium. An obtained culture was inoculated into LB medium containing 50 mg/L kanamycin and 25 mg/L streptomycin, followed by

20 cultivation at 28 °C for about 1 day. Bacterial cells were collected at room temperature, and they were suspended in a suspension medium for infiltration (1/2 MS salt, 1/2 Gamborg B5 vitamin, 5 % sucrose, 0.5 g/L MES, pH 5.7 (KOH), to which, immediately before the use,

25 benzylaminopurine was added to give a final concentration of 0.044 µM, or Silwet L77 was added in an amount of 200 µl per 1 L (final concentration: 0.02 %))

so that OD<sub>600</sub> of an obtained bacterial suspension was 0.8.

Flowers in bloom and fructification were removed from the plants to be subjected to infiltration. The  
5 rock wool was inverted upside down, and flowers which were not in fructification were immersed in the suspension of Agrobacterium, followed by being placed in a desiccator so that the pressure was reduced to be 40 mmHG for 15 minutes. Seeds were harvested after 2 to 4  
10 weeks. The harvested seeds were stored in a desiccator.

Next, transformants were selected on a selection medium. The seeds were sterilized in the same manner as described above, and they were cultivated on a selection medium (MS salt, Gamborg B5 vitamin, 1 % sucrose, 0.5  
15 g/L MES, pH 5.8, 0.8 % agar, to which antibiotics for selection, i.e., carbenicillin (final concentration: 100 mg/L) and kanamycin (final concentration: 50 mg/L) were added after autoclaving)) at 22 °C to select resistant plants. The resistant plants were transferred to a  
20 fresh medium, and they were allowed to grow until true leaves unfolded. Seeds were harvested from the obtained plants. Selection was repeated in the same manner as described above, and thus T3 seeds were obtained. The T3 seeds were measured for the raffinose content in  
25 accordance with the method described above. Results are shown in Table 4.

Table 4

	<u>Plant</u>	<u>Raffinose content (mg/g)</u>
	Wild type	0.2
	Transformant (pBIRS1)	0.0
5	Transformant (pBIRS9)	0.0

Industrial Applicability

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The present invention provides the purified raffinose synthase, the raffinose synthase gene, the chimeric gene comprising the raffinose synthase gene and the regulatory region expressible in plants, and the plant introduced with the chimeric gene.

Raffinose can be efficiently synthesized from sucrose and galactinol by using the raffinose synthase of the present invention. The content of the raffinose family oligosaccharides in plants can be changed by utilizing the raffinose synthase gene or the chimeric gene of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: OSUMI Chieko  
NOZAKI Jinshi  
KIDA Takao
- (ii) TITLE OF INVENTION: RAFFINOSE SYNTHASE GENE, METHOD FOR  
PRODUCING RAFFINOSE, AND TRANSGENIC PLANT
- (iii) NUMBER OF SEQUENCES: 22
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE:
  - (B) STREET:
  - (C) CITY:
  - (E) COUNTRY:
  - (F) ZIP:
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
- (vi) ATTORNEY/AGENT INFORMATION:
  - (A) NAME:
  - (B) REGISTRATION NUMBER:
- (vii) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE:
  - (B) TELEFAX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: internal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Phe	Gly	Trp	Cys	Thr	Trp	Asp	Ala	Phe	Tyr	Leu	Thr	Val	His	Pro	Gln
1				5					10					15	
Gly	Val	Ile	Glu	Gly	Val	Arg	His	Leu	Val	Asp	Gly	Gly	Cys		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 amino acids
  - (B) TYPE: amino acid

(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
Pro Val Ser Val Gly Cys Phe Val Gly Phe Asp Ala Ser Glu Pro Asp  
1 5 10 15  
Ser Arg His

(2) INFORMATION FOR SEQ ID NO:3:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
Tyr Asp Gln Asp Gln Met Val Val Val Gln Val Pro Trp Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2517 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: cDNA to mRNA  
(vi) ORIGINAL SOURCE:  
(A) ORGANISM: cucumber (Cucumis sativas)  
(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 56..2407

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
AAAAACAAC CCTTCTTTTA GTTTTTTGGG TTTGTTTCTT CTTTCTTCT CACAA ATG 58  
Met  
1  
GCT CCT AGT TTT AAA AAT GGT GGC TCC AAC GTA GTT TCA TTT GAT GGC 106  
Ala Pro Ser Phe Lys Asn Gly Gly Ser Asn Val Val Ser Phe Asp Gly  
5 10 15  
TTA AAT GAC ATG TCG TCA CCG TTT GCA ATC GAC GGA TCG GAT TTC ACT 154  
Leu Asn Asp Met Ser Ser Pro Phe Ala Ile Asp Gly Ser Asp Phe Thr  
20 25 30  
GTG AAC GGT CAT TCG TTT CTG TCC GAT GTT CCT GAG AAC ATT GTT GCT 202  
Val Asn Gly His Ser Phe Leu Ser Asp Val Pro Glu Asn Ile Val Ala  
35 40 45  
TCT CCT TCT CCG TAC ACT TCG ATA GAC AAG TCC CCG GTT TCG GTT GGT 250

006260-80252960

Ser	Pro	Ser	Pro	Tyr	Thr	Ser	Ile	Asp	Lys	Ser	Pro	Val	Ser	Val	Gly	
50					55				60						65	
TGC	TTT	GTT	GGA	TTC	GAC	GCG	TCG	GAA	CCT	GAT	AGC	CGA	CAT	GTT	GTT	298
Cys	Phe	Val	Gly	Phe	Asp	Ala	Ser	Glu	Pro	Asp	Ser	Arg	His	Val	Val	
				70					75						80	
TCG	ATT	GGG	AAG	CTG	AAG	GAT	ATT	CGG	TTT	ATG	AGT	ATT	TTC	AGG	TTT	346
Ser	Ile	Gly	Lys	Leu	Lys	Asp	Ile	Arg	Phe	Met	Ser	Ile	Phe	Arg	Phe	
			85					90					95			
AAG	GTT	TGG	TGG	ACT	ACA	CAC	TGG	GTT	GGT	CGA	AAT	GGT	GGG	GAT	CTT	394
Lys	Val	Trp	Trp	Thr	Thr	His	Trp	Val	Gly	Arg	Asn	Gly	Gly	Asp	Leu	
		100					105					110				
GAA	TCG	GAG	ACT	CAG	ATT	GTG	ATC	CTT	GAG	AAG	TCA	GAT	TCT	GGT	CGA	442
Glu	Ser	Glu	Thr	Gln	Ile	Val	Ile	Leu	Glu	Lys	Ser	Asp	Ser	Gly	Arg	
		115				120					125					
CCG	TAT	GTT	TTC	CTT	CTT	CCG	ATC	GTT	GAG	GGA	CCG	TTC	CGA	ACC	TCG	490
Pro	Tyr	Val	Phe	Leu	Leu	Pro	Ile	Val	Glu	Gly	Pro	Phe	Arg	Thr	Ser	
130					135					140					145	
ATT	CAG	CCT	GGG	GAT	GAT	GAC	TTT	GTC	GAT	GTT	TGT	GTC	GAG	AGT	GGT	538
Ile	Gln	Pro	Gly	Asp	Asp	Asp	Phe	Val	Asp	Val	Cys	Val	Glu	Ser	Gly	
				150					155					160		
TCG	TCG	AAA	GTT	GTT	GAT	GCA	TCG	TTC	CGA	AGT	ATG	TTG	TAT	CTT	CAT	586
Ser	Ser	Lys	Val	Val	Asp	Ala	Ser	Phe	Arg	Ser	Met	Leu	Tyr	Leu	His	
			165					170					175			
GCT	GGT	GAT	GAT	CCG	TTT	GCA	CTT	GTT	AAA	GAG	GCG	ATG	AAG	ATC	GTG	634
Ala	Gly	Asp	Asp	Pro	Phe	Ala	Leu	Val	Lys	Glu	Ala	Met	Lys	Ile	Val	
		180				185						190				
AGG	ACC	CAT	CTT	GGA	ACT	TTT	CGC	TTG	TTG	GAG	GAG	AAG	ACT	CCA	CCA	682
Arg	Thr	His	Leu	Gly	Thr	Phe	Arg	Leu	Leu	Glu	Glu	Lys	Thr	Pro	Pro	
		195				200						205				
GGT	ATC	GTG	GAC	AAA	TTC	GGT	TGG	TGC	ACG	TGG	GAC	GCG	TTT	TAC	CTA	730
Gly	Ile	Val	Asp	Lys	Phe	Gly	Trp	Cys	Thr	Trp	Asp	Ala	Phe	Tyr	Leu	
210					215					220					225	
ACG	GTT	CAT	CCA	CAG	GGC	GTA	ATA	GAA	GGC	GTG	AGG	CAT	CTC	GTC	GAC	778
Thr	Val	His	Pro	Gln	Gly	Val	Ile	Glu	Gly	Val	Arg	His	Leu	Val	Asp	
				230					235					240		
GGC	GGT	TGT	CCT	CCC	GGT	TTA	GTC	CTA	ATC	GAC	GAT	GGT	TGG	CAA	TCC	826
Gly	Gly	Cys	Pro	Pro	Gly	Leu	Val	Leu	Ile	Asp	Asp	Gly	Trp	Gln	Ser	
			245					250					255			
ATC	GGA	CAC	GAT	TCG	GAT	CCC	ATC	ACC	AAA	GAA	GGA	ATG	AAC	CAA	ACC	874
Ile	Gly	His	Asp	Ser	Asp	Pro	Ile	Thr	Lys	Glu	Gly	Met	Asn	Gln	Thr	
		260				265						270				
GTC	GCC	GGC	GAG	CAA	ATG	CCC	TGC	CGT	CTT	TTG	AAA	TTC	CAA	GAG	AAT	922
Val	Ala	Gly	Glu	Gln	Met	Pro	Cys	Arg	Leu	Leu	Lys	Phe	Gln	Glu	Asn	
		275				280					285					
TAC	AAA	TTC	CGT	GAC	TAC	GTC	AAT	CCC	AAG	GCC	ACC	GGC	CCC	CGA	GCC	970

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Tyr	Lys	Phe	Arg	Asp	Tyr	Val	Asn	Pro	Lys	Ala	Thr	Gly	Pro	Arg	Ala	
290					295					300					305	
GGC	CAG	AAG	GGG	ATG	AAG	GCG	TTT	ATA	GAT	GAA	CTC	AAA	GGA	GAG	TTT	1018
Gly	Gln	Lys	Gly	Met	Lys	Ala	Phe	Ile	Asp	Glu	Leu	Lys	Gly	Glu	Phe	
			310						315					320		
AAG	ACT	GTG	GAG	CAT	GTT	TAT	GTT	TGG	CAT	GCT	TTG	TGT	GGA	TAT	TGG	1066
Lys	Thr	Val	Glu	His	Val	Tyr	Val	Trp	His	Ala	Leu	Cys	Gly	Tyr	Trp	
			325					330					335			
GGT	GCG	CTT	CGC	CCG	CAG	GTG	CCT	GCG	TTG	CCT	GAG	GCA	CGT	GTG	ATT	1114
Gly	Gly	Leu	Arg	Pro	Gln	Val	Pro	Gly	Leu	Pro	Glu	Ala	Arg	Val	Ile	
		340					345					350				
CAG	CCA	GTG	CTT	TCA	CCA	GGG	CTG	CAG	ATG	ACG	ATG	GAG	GAT	TTG	GCG	1162
Gln	Pro	Val	Leu	Ser	Pro	Gly	Leu	Gln	Met	Thr	Met	Glu	Asp	Leu	Ala	
	355					360					365					
GTG	GAT	AAG	ATT	GTT	CTT	CAT	AAG	GTC	GGG	CTG	GTC	CCG	CCG	GAG	AAG	1210
Val	Asp	Lys	Ile	Val	Leu	His	Lys	Val	Gly	Leu	Val	Pro	Pro	Glu	Lys	
370					375				380						385	
GCT	GAG	GAG	ATG	TAC	GAA	GGA	CTT	CAT	GCT	CAT	TTG	GAA	AAA	GTT	GGG	1258
Ala	Glu	Glu	Met	Tyr	Glu	Gly	Leu	His	Ala	His	Leu	Glu	Lys	Val	Gly	
			390						395					400		
ATC	GAC	GGT	GTT	AAG	ATT	GAC	GTT	ATC	CAC	CTA	TTG	GAG	ATG	TTG	TGT	1306
Ile	Asp	Gly	Val	Lys	Ile	Asp	Val	Ile	His	Leu	Leu	Glu	Met	Leu	Cys	
		405					410					415				
GAA	GAC	TAT	GGA	GGG	AGA	GTG	GAT	TTG	GCA	AAG	GCA	TAT	TAC	AAA	GCA	1354
Glu	Asp	Tyr	Gly	Gly	Arg	Val	Asp	Leu	Ala	Lys	Ala	Tyr	Tyr	Lys	Ala	
	420						425				430					
ATG	ACC	AAA	TCA	ATA	AAT	AAA	CAT	TTT	AAA	GGA	AAT	GGA	GTC	ATT	GCA	1402
Met	Thr	Lys	Ser	Ile	Asn	Lys	His	Phe	Lys	Gly	Asn	Gly	Val	Ile	Ala	
	435					440					445					
AGT	ATG	GAA	CAT	TGT	AAC	GAC	TTC	ATG	TTC	CTT	GGC	ACG	GAA	GCT	ATC	1450
Ser	Met	Glu	His	Cys	Asn	Asp	Phe	Met	Phe	Leu	Gly	Thr	Glu	Ala	Ile	
450					455					460					465	
TCT	CTT	GGT	CGT	GTT	GGT	GAT	GAC	TTT	TGG	TGC	ACG	GAC	CCC	TCT	GGT	1498
Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	Asp	Pro	Ser	Gly	
			470						475					480		
GAT	CCA	AAC	GGT	ACG	TTT	TGG	CTC	CAA	GGA	TGT	CAC	ATG	GTT	CAT	TGT	1546
Asp	Pro	Asn	Gly	Thr	Phe	Trp	Leu	Gln	Gly	Cys	His	Met	Val	His	Cys	
			485					490					495			
GCC	AAC	GAC	AGC	TTG	TGG	ATG	GGG	AAC	TTC	ATC	CAC	CCT	GAC	TGG	GAT	1594
Ala	Asn	Asp	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	His	Pro	Asp	Trp	Asp	
	500						505					510				
ATG	TTC	CAA	TCC	ACC	CAC	CCT	TGT	GCC	GCC	TTC	CAT	GCT	GCC	TCT	CGA	1642
Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Ala	Phe	His	Ala	Ala	Ser	Arg	
	515					520					525					
GCC	ATC	TCT	GGT	GGC	CCG	ATC	TAT	GTT	AGT	GAT	TCT	GTG	GGA	AAG	CAT	1690

006260" 80252950

Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Val	Ser	Asp	Ser	Val	Gly	Lys	His	
530					535					540					545	
AAC	TTT	GAT	CTT	CTG	AAA	AAA	CTA	GTG	CTT	CCT	GAT	GGA	TCG	ATC	CTT	1738
Asn	Phe	Asp	Leu	Leu	Lys	Lys	Leu	Val	Leu	Pro	Asp	Gly	Ser	Ile	Leu	
				550					555						560	
CGA	AGT	GAG	TAC	TAT	GCA	CTC	CCG	ACT	CGC	GAT	TGT	TTG	TTT	GAA	GAC	1786
Arg	Ser	Glu	Tyr	Tyr	Ala	Leu	Pro	Thr	Arg	Asp	Cys	Leu	Phe	Glu	Asp	
			565					570						575		
CCT	TTG	CAT	AAT	GGA	GAA	ACT	ATG	CTT	AAG	ATT	TGG	AAT	CTC	AAC	AAG	1834
Pro	Leu	His	Asn	Gly	Glu	Thr	Met	Leu	Lys	Ile	Trp	Asn	Leu	Asn	Lys	
			580				585							590		
TTC	ACT	GGA	GTG	ATT	GGT	GCA	TTC	AAC	TGC	CAA	GGA	GGA	GGA	TGG	TGT	1882
Phe	Thr	Gly	Val	Ile	Gly	Ala	Phe	Asn	Cys	Gln	Gly	Gly	Gly	Trp	Cys	
	595					600					605					
CGT	GAG	ACA	CGC	CGC	AAC	CAA	TGC	TTT	TCA	CAA	TAC	TCA	AAA	CGA	GTG	1930
Arg	Glu	Thr	Arg	Arg	Asn	Gln	Cys	Phe	Ser	Gln	Tyr	Ser	Lys	Arg	Val	
610					615					620					625	
ACA	TCC	AAA	ACT	AAC	CCA	AAA	GAC	ATA	GAA	TGG	CAC	AGT	GGA	GAA	AAC	1978
Thr	Ser	Lys	Thr	Asn	Pro	Lys	Asp	Ile	Glu	Trp	His	Ser	Gly	Glu	Asn	
				630						635					640	
CCT	ATC	TCT	ATT	GAA	GGC	GTT	AAA	ACC	TTT	GCG	CTT	TAC	CTC	TAT	CAA	2026
Pro	Ile	Ser	Ile	Glu	Gly	Val	Lys	Thr	Phe	Ala	Leu	Tyr	Leu	Tyr	Gln	
			645					650						655		
GCC	AAA	AAA	CTT	ATC	CTC	TCC	AAG	CCC	TCT	CAA	GAT	CTT	GAC	ATA	GCT	2074
Ala	Lys	Lys	Leu	Ile	Leu	Ser	Lys	Pro	Ser	Gln	Asp	Leu	Asp	Ile	Ala	
			660				665							670		
CTT	GAC	CCA	TTC	GAA	TTC	GAG	CTC	ATC	ACT	GTT	TCA	CCA	GTG	ACC	AAA	2122
Leu	Asp	Pro	Phe	Glu	Phe	Glu	Leu	Ile	Thr	Val	Ser	Pro	Val	Thr	Lys	
			675				680							685		
CTC	ATC	CAA	ACT	TCT	CTA	CAC	TTT	GCC	CCA	ATT	GGG	CTG	GTG	AAC	ATG	2170
Leu	Ile	Gln	Thr	Ser	Leu	His	Phe	Ala	Pro	Ile	Gly	Leu	Val	Asn	Met	
690					695					700					705	
CTT	AAC	ACT	AGT	GGA	GCC	ATC	CAA	TCT	GTG	GAC	TAT	GAC	GAT	GAC	CTA	2218
Leu	Asn	Thr	Ser	Gly	Ala	Ile	Gln	Ser	Val	Asp	Tyr	Asp	Asp	Asp	Leu	
				710						715					720	
AGC	TCA	GTC	GAG	ATT	GGT	GTC	AAA	GGG	TGT	GGT	GAG	ATG	CGA	GTA	TTT	2266
Ser	Ser	Val	Glu	Ile	Gly	Val	Lys	Gly	Cys	Gly	Glu	Met	Arg	Val	Phe	
			725						730						735	
GCA	TCG	AAA	AAA	CCA	AGG	GCT	TGT	CGT	ATT	GAT	GGG	GAG	GAT	GTT	GGG	2314
Ala	Ser	Lys	Lys	Pro	Arg	Ala	Cys	Arg	Ile	Asp	Gly	Glu	Asp	Val	Gly	
			740				745							750		
TTC	AAG	TAT	GAT	CAG	GAC	CAA	ATG	GTG	GTG	GTT	CAA	GTG	CCA	TGG	CCA	2362
Phe	Lys	Tyr	Asp	Gln	Asp	Gln	Met	Val	Val	Val	Gln	Val	Pro	Trp	Pro	
			755				760								765	
ATT	GAT	TCT	TCA	TCG	GGT	GGC	ATT	TCG	GTT	ATC	GAG	TAC	TTG	TTT		2407

005260" 80252960

Ile Asp Ser Ser Ser Gly Gly Ile Ser Val Ile Glu Tyr Leu Phe  
 770 775 780  
 TAATTTTAT TTATGTAAGC TCAATGATTG TTGTTGTTGT CGCTGTTGTT GCTATCAATG 2467  
 TATTTCTCTC CAAAAGAAAA TTATGTGTAA TTTGGAGAGT AATTAAGTGA 2517

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 784 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Ala	Pro	Ser	Phe	Lys	Asn	Gly	Gly	Ser	Asn	Val	Val	Ser	Phe	Asp
1				5					10					15	
Gly	Leu	Asn	Asp	Met	Ser	Ser	Pro	Phe	Ala	Ile	Asp	Gly	Ser	Asp	Phe
			20					25					30		
Thr	Val	Asn	Gly	His	Ser	Phe	Leu	Ser	Asp	Val	Pro	Glu	Asn	Ile	Val
			35				40					45			
Ala	Ser	Pro	Ser	Pro	Tyr	Thr	Ser	Ile	Asp	Lys	Ser	Pro	Val	Ser	Val
			50			55				60					
Gly	Cys	Phe	Val	Gly	Phe	Asp	Ala	Ser	Glu	Pro	Asp	Ser	Arg	His	Val
65					70				75					80	
Val	Ser	Ile	Gly	Lys	Leu	Lys	Asp	Ile	Arg	Phe	Met	Ser	Ile	Phe	Arg
				85					90					95	
Phe	Lys	Val	Trp	Trp	Thr	Thr	His	Trp	Val	Gly	Arg	Asn	Gly	Gly	Asp
			100				105					110			
Leu	Glu	Ser	Glu	Thr	Gln	Ile	Val	Ile	Leu	Glu	Lys	Ser	Asp	Ser	Gly
			115				120					125			
Arg	Pro	Tyr	Val	Phe	Leu	Leu	Pro	Ile	Val	Glu	Gly	Pro	Phe	Arg	Thr
			130			135				140					
Ser	Ile	Gln	Pro	Gly	Asp	Asp	Asp	Phe	Val	Asp	Val	Cys	Val	Glu	Ser
145					150				155					160	
Gly	Ser	Ser	Lys	Val	Val	Asp	Ala	Ser	Phe	Arg	Ser	Met	Leu	Tyr	Leu
			165					170					175		
His	Ala	Gly	Asp	Asp	Pro	Phe	Ala	Leu	Val	Lys	Glu	Ala	Met	Lys	Ile
			180				185					190			
Val	Arg	Thr	His	Leu	Gly	Thr	Phe	Arg	Leu	Leu	Glu	Glu	Lys	Thr	Pro
			195				200					205			
Pro	Gly	Ile	Val	Asp	Lys	Phe	Gly	Trp	Cys	Thr	Trp	Asp	Ala	Phe	Tyr
			210			215					220				
Leu	Thr	Val	His	Pro	Gln	Gly	Val	Ile	Glu	Gly	Val	Arg	His	Leu	Val
225					230				235					240	
Asp	Gly	Gly	Cys	Pro	Pro	Gly	Leu	Val	Leu	Ile	Asp	Asp	Gly	Trp	Gln
			245					250					255		
Ser	Ile	Gly	His	Asp	Ser	Asp	Pro	Ile	Thr	Lys	Glu	Gly	Met	Asn	Gln

006260" 80257960

			260					265				270			
Thr	Val	Ala	Gly	Glu	Gln	Met	Pro	Cys	Arg	Leu	Leu	Lys	Phe	Gln	Glu
		275					280					285			
Asn	Tyr	Lys	Phe	Arg	Asp	Tyr	Val	Asn	Pro	Lys	Ala	Thr	Gly	Pro	Arg
	290					295					300				
Ala	Gly	Gln	Lys	Gly	Met	Lys	Ala	Phe	Ile	Asp	Glu	Leu	Lys	Gly	Glu
305					310					315					320
Phe	Lys	Thr	Val	Glu	His	Val	Tyr	Val	Trp	His	Ala	Leu	Cys	Gly	Tyr
				325					330					335	
Trp	Gly	Gly	Leu	Arg	Pro	Gln	Val	Pro	Gly	Leu	Pro	Glu	Ala	Arg	Val
			340					345					350		
Ile	Gln	Pro	Val	Leu	Ser	Pro	Gly	Leu	Gln	Met	Thr	Met	Glu	Asp	Leu
	355						360					365			
Ala	Val	Asp	Lys	Ile	Val	Leu	His	Lys	Val	Gly	Leu	Val	Pro	Pro	Glu
	370					375					380				
Lys	Ala	Glu	Glu	Met	Tyr	Glu	Gly	Leu	His	Ala	His	Leu	Glu	Lys	Val
385					390					395					400
Gly	Ile	Asp	Gly	Val	Lys	Ile	Asp	Val	Ile	His	Leu	Leu	Glu	Met	Leu
				405					410					415	
Cys	Glu	Asp	Tyr	Gly	Gly	Arg	Val	Asp	Leu	Ala	Lys	Ala	Tyr	Tyr	Lys
		420						425					430		
Ala	Met	Thr	Lys	Ser	Ile	Asn	Lys	His	Phe	Lys	Gly	Asn	Gly	Val	Ile
	435					440						445			
Ala	Ser	Met	Glu	His	Cys	Asn	Asp	Phe	Met	Phe	Leu	Gly	Thr	Glu	Ala
	450					455					460				
Ile	Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	Asp	Pro	Ser
465					470					475					480
Gly	Asp	Pro	Asn	Gly	Thr	Phe	Trp	Leu	Gln	Gly	Cys	His	Met	Val	His
				485					490					495	
Cys	Ala	Asn	Asp	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	His	Pro	Asp	Trp
		500						505					510		
Asp	Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Ala	Phe	His	Ala	Ala	Ser
	515						520					525			
Arg	Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Val	Ser	Asp	Ser	Val	Gly	Lys
	530					535					540				
His	Asn	Phe	Asp	Leu	Leu	Lys	Lys	Leu	Val	Leu	Pro	Asp	Gly	Ser	Ile
545					550					555					560
Leu	Arg	Ser	Glu	Tyr	Tyr	Ala	Leu	Pro	Thr	Arg	Asp	Cys	Leu	Phe	Glu
			565						570					575	
Asp	Pro	Leu	His	Asn	Gly	Glu	Thr	Met	Leu	Lys	Ile	Trp	Asn	Leu	Asn
		580						585				590			
Lys	Phe	Thr	Gly	Val	Ile	Gly	Ala	Phe	Asn	Cys	Gln	Gly	Gly	Gly	Trp
	595						600					605			
Cys	Arg	Glu	Thr	Arg	Arg	Asn	Gln	Cys	Phe	Ser	Gln	Tyr	Ser	Lys	Arg
	610					615						620			

006260"80252960

Val	Thr	Ser	Lys	Thr	Asn	Pro	Lys	Asp	Ile	Glu	Trp	His	Ser	Gly	Glu
625					630					635					640
Asn	Pro	Ile	Ser	Ile	Glu	Gly	Val	Lys	Thr	Phe	Ala	Leu	Tyr	Leu	Tyr
			645					650						655	
Gln	Ala	Lys	Lys	Leu	Ile	Leu	Ser	Lys	Pro	Ser	Gln	Asp	Leu	Asp	Ile
		660					665						670		
Ala	Leu	Asp	Pro	Phe	Glu	Phe	Glu	Leu	Ile	Thr	Val	Ser	Pro	Val	Thr
	675						680					685			
Lys	Leu	Ile	Gln	Thr	Ser	Leu	His	Phe	Ala	Pro	Ile	Gly	Leu	Val	Asn
690						695					700				
Met	Leu	Asn	Thr	Ser	Gly	Ala	Ile	Gln	Ser	Val	Asp	Tyr	Asp	Asp	Asp
705					710					715					720
Leu	Ser	Ser	Val	Glu	Ile	Gly	Val	Lys	Gly	Cys	Gly	Glu	Met	Arg	Val
			725					730						735	
Phe	Ala	Ser	Lys	Lys	Pro	Arg	Ala	Cys	Arg	Ile	Asp	Gly	Glu	Asp	Val
		740					745					750			
Gly	Phe	Lys	Tyr	Asp	Gln	Asp	Gln	Met	Val	Val	Val	Gln	Val	Pro	Trp
	755					760					765				
Pro	Ile	Asp	Ser	Ser	Ser	Gly	Gly	Ile	Ser	Val	Ile	Glu	Tyr	Leu	Phe
770						775					780				

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc= "Synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTYTAYCTBA CHGTNCAYCC TCA

23

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc= "Synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTYTAYCTBA CHGTNCAYCC CCA

23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
TTYTAYCTBA CHGTNCAYCC ACA 23

(2) INFORMATION FOR SEQ ID NO:9:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:  
TTYTAYCTBA CHGTNCAYCC GCA 23

(2) INFORMATION FOR SEQ ID NO:10:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 6 and 11 = inosine  
Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
GARGGNGTNM GNCAYCTRGT NGAYGG 26

(2) INFORMATION FOR SEQ ID NO:11:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:

006260" 80257960

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION: N at 6 and 11 = inosine

Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GARGGNGTNM GNCAYCTYGT NGAYGG

26

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION: N at 6 and 11 = inosine

Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GARGGNGTNM GNCAYTTRGT NGAYGG

26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION: N at 3 = inosine

Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTNGGNTGYT TYGTNGGYTT YGAYGC

26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

006260" 80252950

- (ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"
- (ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 3 = inosine  
Other N = A, G, C, or T
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:  
GTNGGNTGYT TYGTNGGRTT YGAYGC

26

- (2) INFORMATION FOR SEQ ID NO:15:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"
- (ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 9 and 11 = inosine  
Other N = A, G, C, or T
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:  
TTYGAYGCNT CNGARCCHGA YTCDCGNCA

29

- (2) INFORMATION FOR SEQ ID NO:16:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"
- (ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 9 and 11 = inosine  
Other N = A, G, C, or T
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:  
TTYGAYGCNT CNGARCCHGA YTCDAGYCA

30

- (2) INFORMATION FOR SEQ ID NO:17:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:  
GAYCARGAYC TRATGGTNGT 20

(2) INFORMATION FOR SEQ ID NO:18:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 6 and 15 = inosine  
Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:  
CCRTCNACYA GRTGNCKNAC NCCYTC 26

(2) INFORMATION FOR SEQ ID NO:19:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 6 and 15 = inosine  
Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:  
CCRTCNACRA GRTGNCKNAC NCCYTC 26

(2) INFORMATION FOR SEQ ID NO:20:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single

006260"8025/960

(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 6 and 15 = inosine  
Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:  
CCRTCACACG TRGTGCKACG NCCATC

26

(2) INFORMATION FOR SEQ ID NO:21:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 3 and 18 = inosine  
Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:  
TGACGCGATG CDGGATCAGG NGCATCAG

29

(2) INFORMATION FOR SEQ ID NO:22:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
(A) NAME/KEY:  
(B) LOCATION:  
(D) OTHER INFORMATION: N at 19 = inosine  
Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:  
ATGCTGATGAT TCDGGATCAGG NGCATCAG

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What is claimed is:

1. A raffinose synthase which has the following properties:

(1) action and substrate specificity: the raffinose synthase produces raffinose from sucrose and galactinol;

(2) optimum pH: the raffinose synthase has an optimum pH of about 6 to 8;

(3) optimum temperature: the raffinose synthase has an optimum temperature of about 35 to 40 °C;

(4) molecular weight: the raffinose synthase has:

(i) a molecular weight of about 75 kDa to 95 kDa estimated by gel filtration chromatography;

(ii) a molecular weight of about 90 kDa to 100 kDa estimated by polyacrylamide gel electrophoresis; and

(iii) a molecular weight of about 90 kDa to 100 kDa estimated by SDS-polyacrylamide gel electrophoresis under a reduced condition;

(5) inhibition: the raffinose synthase is inhibited by iodoacetamide, N-ethylmaleimide, and myo-inositol.

2. The raffinose synthase according to claim 1, which has an amino acid sequence including respective amino acid sequences shown in SEQ ID NOs. 1 to 3 in Sequence Listing.

3. A raffinose synthase which is a protein specified by the following item (A) or (B):

006260-8025/960

(A) a protein which has an amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing; or

(B) a protein which comprises an amino acid sequence including substitution, deletion, insertion, addition, or inversion of one or several residues of amino acids in the amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing, and which has an activity to produce raffinose from sucrose and galactinol.

4. A method for producing raffinose, comprising the step of allowing the raffinose synthase as defined in any one of claims 1 to 3 to act on sucrose and galactinol to produce raffinose.

5. DNA encoding raffinose synthase as defined in any one of claims 1 to 3.

6. DNA coding for a protein specified by the following item (A) or (B):

(A) a protein which has an amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing; or

(B) a protein which comprises an amino acid sequence including substitution, deletion, insertion, addition, or inversion of one or several residues of amino acids in the amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing, and which has an activity to produce raffinose from sucrose and galactinol.

7. The DNA according to claim 5, which is DNA specified by the following item (a) or (b):

(a) DNA which includes a nucleotide sequence

006260-80292960

comprising at least nucleotide residues having nucleotide numbers of 57 to 2408 in a nucleotide sequence shown in SEQ ID NO: 4 in Sequence Listing; or

(b) DNA which is hybridizable under a stringent condition with the nucleotide sequence comprising at least nucleotide residues having nucleotide numbers of 57 to 2408 in the nucleotide sequence shown in SEQ ID NO: 4 in Sequence Listing, and which codes for a protein having an activity to produce raffinose from sucrose and galactinol.

8. A chimeric gene comprising a raffinose synthase gene or a part thereof, and a transcription regulatory region expressible in plant cells.

9. The chimeric gene according to claim 8, wherein the raffinose synthase gene is DNA as defined in any one of claims 5 to 7.

10. The chimeric gene according to claim 8 or 9, wherein the transcription regulatory region is ligated with the DNA so that antisense RNA having a sequence complementary to a coding strand of the DNA is expressed.

11. A plant which is transformed with the chimeric gene as defined in any one of claims 8 to 10.

12. A method for changing a content of raffinose family oligosaccharides in a plant, comprising the steps of transforming the plant with the chimeric gene as defined in any one of claims 8 to 10, and expressing the

005260-8025/960

- 82 -

gene in cells of the plant.

006260" 8029/850

Abstract

Raffinose synthase purified from cucumber is allowed to act on sucrose and galactinol. Thus raffinose is efficiently produced. The function of endogenous raffinose synthase is regulated by transforming a plant with a chimeric gene comprising a raffinose synthase gene and a regulatory region expressible in the plant. Thus a plant, in which raffinose family oligosaccharides are decreased, is created.

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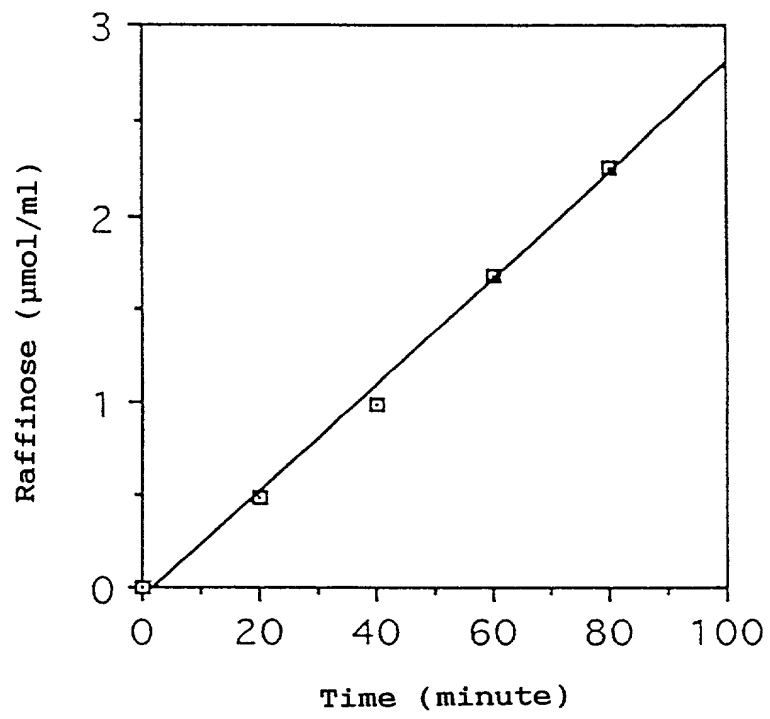


FIG. 1

M S

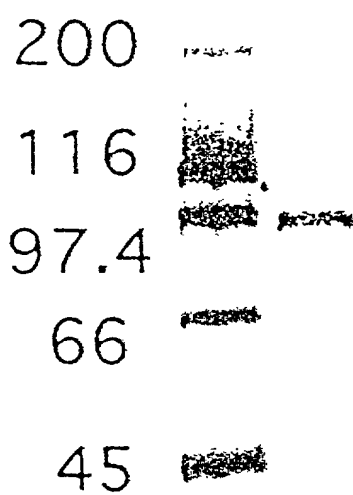


FIG. 2

006260" 8025/960

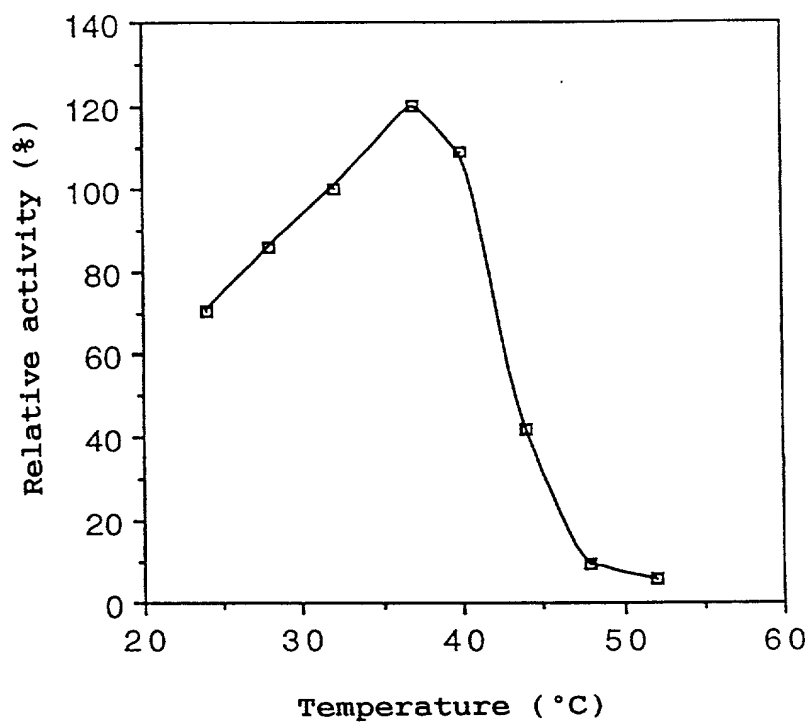


FIG. 3

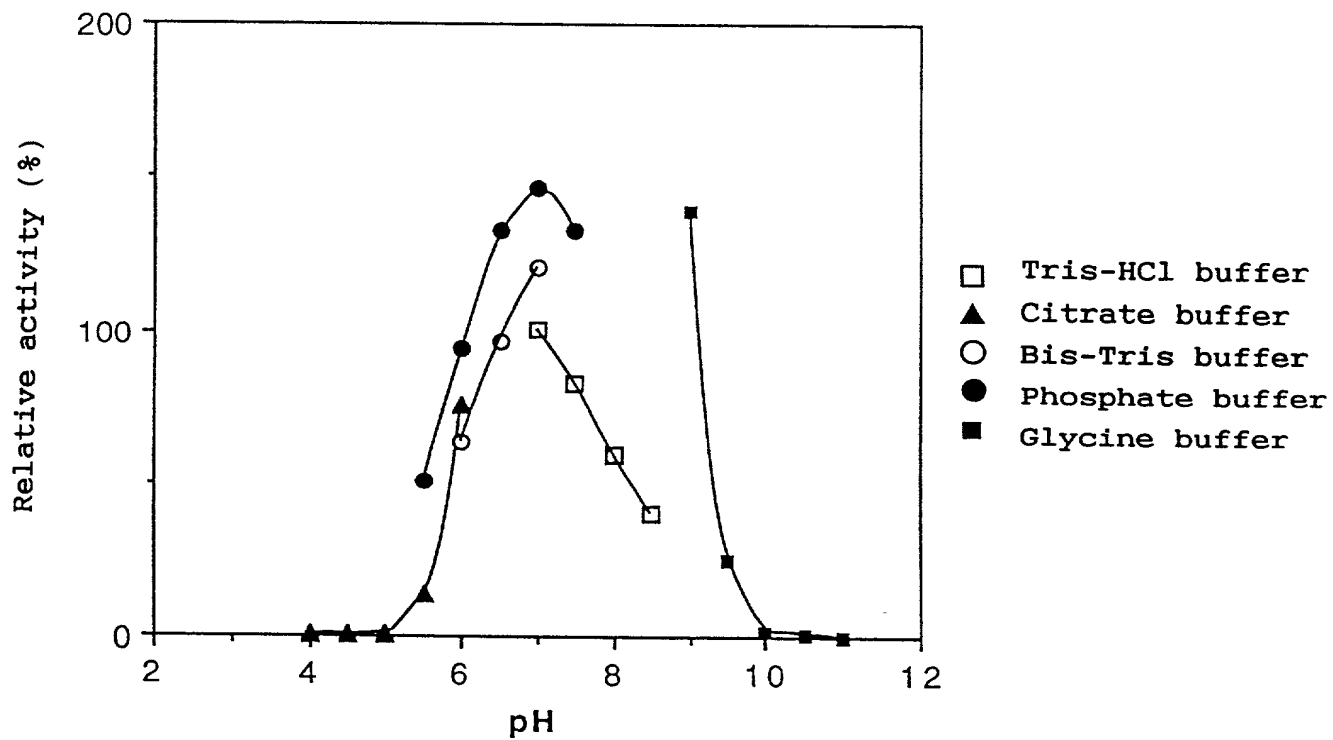


FIG. 4

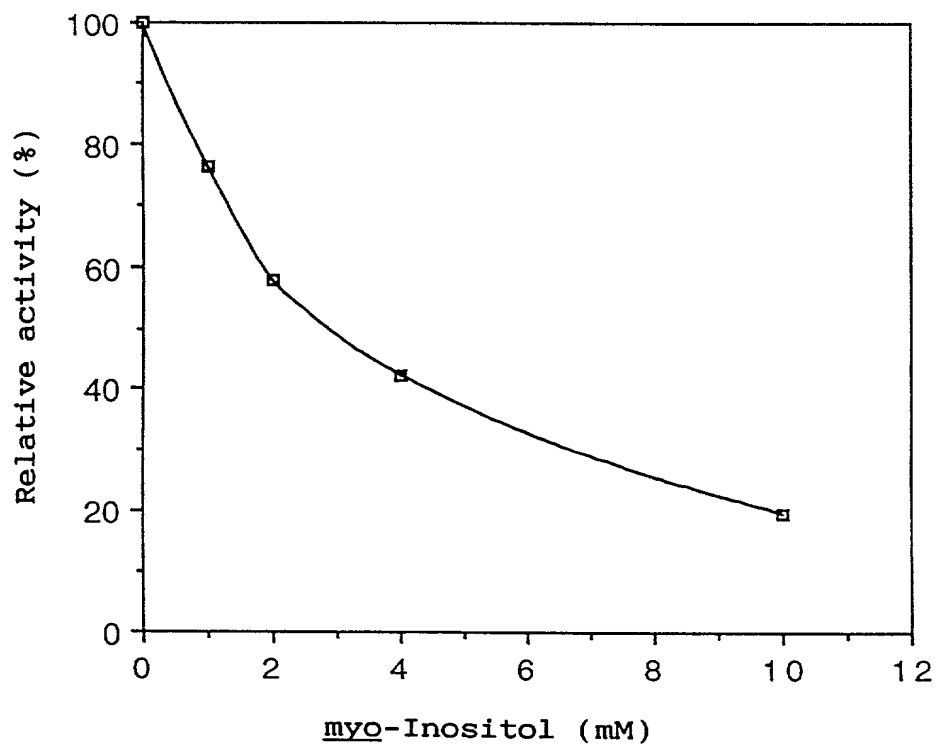


FIG. 5

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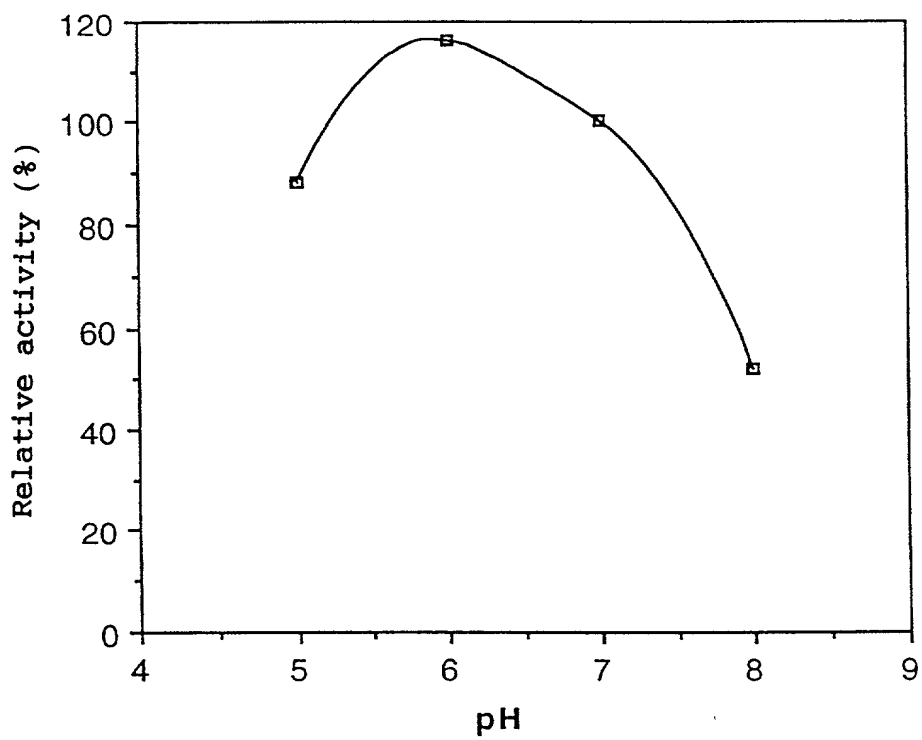


FIG. 6

(SEQ ID NO:6) A1 5' - TTY TAY CTB ACH GTN CAY CCT CA -3'  
 (SEQ ID NO:7) A2 5' - TTY TAY CTB ACH GTN CAY CCC CA -3' B1 5' - GAR GGN GTN MGN CAY CTR GTN GAY GG -3' (SEQ ID NO:10)  
 (SEQ ID NO:8) A3 5' - TTY TAY CTB ACH GTN CAY CCA CA -3' B2 5' - GAR GGN GTN MGN CAY CTY GTN GAY GG -3' (SEQ ID NO:11)  
 (SEQ ID NO:9) A4 5' - TTY TAY CTB ACH GTN CAY CCG CA -3' B3 5' - GAR GGN GTN MGN CAY TTR GTN GAY GG -3' (SEQ ID NO:12)  
 Phe Gly Trp Cys Thr Trp Asp Ala Phe Tyr Leu Thr Val His Pro Gln Gly Val Ile Glu Gly Val Arg His Leu Val Asp Gly Gly Cys  
 (SEQ ID NO:1) (SEQ ID NO:18) 3' - CTY CCN CAN KCI GTR GAY CAI CTR CC -5' B' 1  
 (SEQ ID NO:19) 3' - CTY CCN CAN KCI GTR GAR CAI CTR CC -5' B' 2  
 (SEQ ID NO:20) 3' - CTY CCN CAN KCI GTR TAY CAI CTR CC -5' B' 3

D1 5' - TTY GAY GCN TCN GAR CCH GAY TCD CGN CA -3' (SEQ ID NO:15)  
 D2 5' - TTY GAY GCN TCN GAR CCH GAY TCD AGY CAY -3' (SEQ ID NO:16)  
 C1 5' - GTN GGN TGY TTY GTN GGY TTY GAY GC -3' (SEQ ID NO:13)  
 C2 5' - GTN GGN TGY TTY GTN GGR TTY GAY GC -3' (SEQ ID NO:14)  
 Pro Val Ser Val Gly Cys Phe Val Gly Phe Asp Ala Ser Glu Pro Asp Ser Arg His  
 (SEQ ID NO:2) 3' - AAR CTR CGN AGI CTY GGD CTR AGH GCI GT -5' D' 1 (SEQ ID NO:21)  
 3' - AAR CTR CGN AGI CTY GGD CTR AGH TCR GTR -5' D' 2 (SEQ ID NO:22)

E 5' - GAY CAR GAY CTR ATG GTN GT -3' (SEQ ID NO:17)  
 Tyr Asp Gln Asp Gln Met Val Val Val Gln Val Pro Trp Pro  
 (SEQ ID NO:3)

# Declaration, Power Of Attorney and Petition

Page 1 of 3

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

RAFFINOSE SYNTHASE GENE, METHOD FOR PRODUCING

RAFFINOSE, AND TRANSGENIC PLANT

the specification of which

☐ is attached hereto.

☒ was filed on April 28, 1997 as

Application Serial No. 08/846,234

and amended on

☐ was filed as PCT international application

Number

on

and was amended under PCT Article 19

on (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
8-107682	Japan	26/04/1996	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
8-198079	Japan	26/07/1996	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

_____	_____
(Application Number)	(Filing Date)
_____	_____
(Application Number)	(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

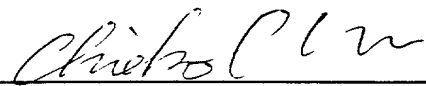
Application Serial No.	Filing Date	Status (pending, patented, abandoned)
_____	_____	_____
_____	_____	_____
_____	_____	_____

And we (I) hereby appoint: Norman F. Oblon, Registration Number 24,618; Marvin J. Spivak, Registration Number 24,913; C. Irvin McClelland, Registration Number 21,124; Gregory J. Maier, Registration Number 25,599; Arthur I. Neustadt, Registration Number 24,854; Richard D. Kelly, Registration Number 27,757; James D. Hamilton, Registration Number 28,421; Eckhard H. Kuesters, Registration Number 28,870; Robert T. Pous, Registration Number 29,099; Charles L. Gholz, Registration Number 26,395; Vincent J. Sunderdick, Registration Number 29,004; William E. Beaumont, Registration Number 30,996; Steven B. Kelber, Registration Number 30,073; Robert F. Gnuse, Registration Number 27,295; Jean-Paul Lavalleye, Registration Number 31,451; Timothy R. Schwartz, Registration Number 32,171; Stephen G. Baxter, Registration Number 32,884; Martin M. Zoltick, Registration Number 35,745; Robert W. Hahl, Registration Number 33,893; Richard L. Treanor, Registration Number 36,379; Steven P. Weihrouch, Registration Number 32,829; John T. Goolkasian, Registration Number 26,142; Marc R. Labgold, Registration Number 34,651; William J. Healey, Registration Number 36,160; Richard L. Chinn, Registration Number 34,305; Steven E. Lipman, Registration Number 30,011; Carl E. Schlier, Registration Number 34,426; James J. Kulbaski, Registration Number 34,648; Catherine B. Richardson, Registration Number 39,007; Richard A. Neifeld, Registration Number 35,299; J. Derek Mason, Registration Number 35,270; and Jacques M. Dulin, Registration Number 24,067; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C., whose Post Office Address is: Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Signature of Inventor

Citizen of: Japan

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September 3, 1997  
Date

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NAME OF SECOND JOINT INVENTOR

  
Signature of Inventor

September 3, 1997  
Date

Takao Kida  
NAME OF THIRD JOINT INVENTOR

  
Signature of Inventor

September 3, 1997  
Date

NAME OF FOURTH JOINT INVENTOR

Signature of Inventor

Date

NAME OF FIFTH JOINT INVENTOR

Signature of Inventor

Date

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# SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: OSUMI Chieko  
NOZAKI Jinshi  
KIDA Takao
- (ii) TITLE OF INVENTION: RAFFINOSE SYNTHASE GENE, METHOD FOR  
PRODUCING RAFFINOSE, AND TRANSGENIC PLANT
- (iii) NUMBER OF SEQUENCES: 22
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: OBLON, SPIVAK, MCCLELLAND, MAIER & NEUSTADT, P.C.
  - (B) STREET: 1755 S. JEFFERSON DAVIS HIGHWAY, FOURTH FLOOR
  - (C) CITY: ARLINGTON
  - (D) STATE: VIRGINIA
  - (E) COUNTRY: USA
  - (F) ZIP: 22202
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
- (vi) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: NORMAN F. OBLON
  - (B) REGISTRATION NUMBER: 24,618
- (vii) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (703)-413-3000
  - (B) TELEFAX: (703)-413-2220

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Phe	Gly	Trp	Cys	Thr	Trp	Asp	Ala	Phe	Tyr	Leu	Thr	Val	His	Pro	Gln
1				5					10					15	
Gly	Val	Ile	Glu	Gly	Val	Arg	His	Leu	Val	Asp	Gly	Gly	Cys		
		20				25							30		

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

Pro Val Ser Val Gly Cys Phe Val Gly Phe Asp Ala Ser Glu Pro Asp  
1 5 10 15  
Ser Arg His

Tyr Asp Gln Asp Gln Met Val Val Val Gln Val Pro Trp Pro  
1 5 10

AAAAACAAC CCTTCTTTTA GTTTTTGGG TTTGTTTCTT CTTTCTTCT CACAA ATG 58  
Met  
1

GCT	CCT	AGT	TTT	AAA	AAT	GGT	GGC	TCC	AAC	GTA	GTT	TCA	TTT	GAT	GGC	106
Ala	Pro	Ser	Phe	Lys	Asn	Gly	Gly	Ser	Asn	Val	Val	Ser	Phe	Asp	Gly	
			5				10			15						
TTA	AAT	GAC	ATG	TCG	TCA	CCG	TTT	GCA	ATC	GAC	GGA	TCG	GAT	TTC	ACT	154
Leu	Asn	Asp	Met	Ser	Ser	Pro	Phe	Ala	Ile	Asp	Gly	Ser	Asp	Phe	Thr	
			20				25			30						
GTG	AAC	GGT	CAT	TCG	TTT	CTG	TCC	GAT	GTT	CCT	GAG	AAC	ATT	GTT	GCT	202
Val	Asn	Gly	His	Ser	Phe	Leu	Ser	Asp	Val	Pro	Glu	Asn	Ile	Val	Ala	
			35				40			45						
TCT	CCT	TCT	CCG	TAC	ACT	TCG	ATA	GAC	AAG	TCC	CCG	GTT	TCG	GTT	GGT	250
Ser	Pro	Ser	Pro	Tyr	Thr	Ser	Ile	Asp	Lys	Ser	Pro	Val	Ser	Val	Gly	
			50				55			60			65			
TGC	TTT	GTT	GGA	TTC	GAC	GCG	TCG	GAA	CCT	GAT	AGC	CGA	CAT	GTT	GTT	298
Cys	Phe	Val	Gly	Phe	Asp	Ala	Ser	Glu	Pro	Asp	Ser	Arg	His	Val	Val	
			70				75			80						
TCG	ATT	GGG	AAG	CTG	AAG	GAT	ATT	CGG	TTT	ATG	AGT	ATT	TTC	AGG	TTT	346
Ser	Ile	Gly	Lys	Leu	Lys	Asp	Ile	Arg	Phe	Met	Ser	Ile	Phe	Arg	Phe	

85							90					95					
AAG	GTT	TGG	TGG	ACT	ACA	CAC	TGG	GTT	GGT	CGA	AAT	GGT	GGG	GAT	CTT	394	
Lys	Val	Trp	Trp	Thr	Thr	His	Trp	Val	Gly	Arg	Asn	Gly	Gly	Asp	Leu		
100							105					110					
GAA	TCG	GAG	ACT	CAG	ATT	GTG	ATC	CTT	GAG	AAG	TCA	GAT	TCT	GGT	CGA	442	
Glu	Ser	Glu	Thr	Gln	Ile	Val	Ile	Leu	Glu	Lys	Ser	Asp	Ser	Gly	Arg		
115							120					125					
CCG	TAT	GTT	TTC	CTT	CTT	CCG	ATC	GTT	GAG	GGA	CCG	TTC	CGA	ACC	TCG	490	
Pro	Tyr	Val	Phe	Leu	Leu	Pro	Ile	Val	Glu	Gly	Pro	Phe	Arg	Thr	Ser		
130							135					140					
ATT	CAG	CCT	GGG	GAT	GAT	GAC	TTT	GTC	GAT	GTT	TGT	GTC	GAG	AGT	GGT	538	
Ile	Gln	Pro	Gly	Asp	Asp	Asp	Phe	Val	Asp	Val	Cys	Val	Glu	Ser	Gly		
150							155					160					
TCG	TCG	AAA	GTT	GTT	GAT	GCA	TCG	TTC	CGA	AGT	ATG	TTG	TAT	CTT	CAT	586	
Ser	Ser	Lys	Val	Val	Asp	Ala	Ser	Phe	Arg	Ser	Met	Leu	Tyr	Leu	His		
165							170					175					
GCT	GGT	GAT	GAT	CCG	TTT	GCA	CTT	GTT	AAA	GAG	GCG	ATG	AAG	ATC	GTG	634	
Ala	Gly	Asp	Asp	Pro	Phe	Ala	Leu	Val	Lys	Glu	Ala	Met	Lys	Ile	Val		
180							185					190					
AGG	ACC	CAT	CTT	GGA	ACT	TTT	CGC	TTG	TTG	GAG	GAG	AAG	ACT	CCA	CCA	682	
Arg	Thr	His	Leu	Gly	Thr	Phe	Arg	Leu	Leu	Glu	Glu	Lys	Thr	Pro	Pro		
195							200					205					
GGT	ATC	GTG	GAC	AAA	TTC	GGT	TGG	TGC	ACG	TGG	GAC	GCG	TTT	TAC	CTA	730	
Gly	Ile	Val	Asp	Lys	Phe	Gly	Trp	Cys	Thr	Trp	Asp	Ala	Phe	Tyr	Leu		
210							215					220					
ACG	GTT	CAT	CCA	CAG	GGC	GTA	ATA	GAA	GGC	GTG	AGG	CAT	CTC	GTC	GAC	778	
Thr	Val	His	Pro	Gln	Gly	Val	Ile	Glu	Gly	Val	Arg	His	Leu	Val	Asp		
230							235					240					
GGC	GGT	TGT	CCT	CCC	GGT	TTA	GTC	CTA	ATC	GAC	GAT	GGT	TGG	CAA	TCC	826	
Gly	Gly	Cys	Pro	Pro	Gly	Leu	Val	Leu	Ile	Asp	Asp	Gly	Trp	Gln	Ser		
245							250					255					
ATC	GGA	CAC	GAT	TCG	GAT	CCC	ATC	ACC	AAA	GAA	GGA	ATG	AAC	CAA	ACC	874	
Ile	Gly	His	Asp	Ser	Asp	Pro	Ile	Thr	Lys	Glu	Gly	Met	Asn	Gln	Thr		
260							265					270					
GTC	GCC	GGC	GAG	CAA	ATG	CCC	TGC	CGT	CTT	TTG	AAA	TTC	CAA	GAG	AAT	922	
Val	Ala	Gly	Glu	Gln	Met	Pro	Cys	Arg	Leu	Leu	Lys	Phe	Gln	Glu	Asn		
275							280					285					
TAC	AAA	TTC	CGT	GAC	TAC	GTC	AAT	CCC	AAG	GCC	ACC	GGC	CCC	CGA	GCC	970	
Tyr	Lys	Phe	Arg	Asp	Tyr	Val	Asn	Pro	Lys	Ala	Thr	Gly	Pro	Arg	Ala		
290							295					300					
GGC	CAG	AAG	GGG	ATG	AAG	GCG	TTT	ATA	GAT	GAA	CTC	AAA	GGA	GAG	TTT	1018	
Gly	Gln	Lys	Gly	Met	Lys	Ala	Phe	Ile	Asp	Glu	Leu	Lys	Gly	Glu	Phe		
310							315					320					
AAG	ACT	GTG	GAG	CAT	GTT	TAT	GTT	TGG	CAT	GCT	TTG	TGT	GGA	TAT	TGG	1066	
Lys	Thr	Val	Glu	His	Val	Tyr	Val	Trp	His	Ala	Leu	Cys	Gly	Tyr	Trp		
325							330					335					
GGT	GGC	CTT	CGC	CCG	CAG	GTG	CCT	GGC	TTG	CCT	GAG	GCA	CGT	GTG	ATT	1114	
Gly	Gly	Leu	Arg	Pro	Gln	Val	Pro	Gly	Leu	Pro	Glu	Ala	Arg	Val	Ile		
340							345					350					

CAG	CCA	GTG	CTT	TCA	CCA	GGG	CTG	CAG	ATG	ACG	ATG	GAG	GAT	TTG	GCG	1162
Gln	Pro	Val	Leu	Ser	Pro	Gly	Leu	Gln	Met	Thr	Met	Glu	Asp	Leu	Ala	
355						360					365					
GTG	GAT	AAG	ATT	GTT	CTT	CAT	AAG	GTC	GGG	CTG	GTC	CCG	CCG	GAG	AAG	1210
Val	Asp	Lys	Ile	Val	Leu	His	Lys	Val	Gly	Leu	Val	Pro	Pro	Glu	Lys	
370					375					380					385	
GCT	GAG	GAG	ATG	TAC	GAA	GGA	CTT	CAT	GCT	CAT	TTG	GAA	AAA	GTT	GGG	1258
Ala	Glu	Glu	Met	Tyr	Glu	Gly	Leu	His	Ala	His	Leu	Glu	Lys	Val	Gly	
				390					395					400		
ATC	GAC	GGT	GTT	AAG	ATT	GAC	GTT	ATC	CAC	CTA	TTG	GAG	ATG	TTG	TGT	1306
Ile	Asp	Gly	Val	Lys	Ile	Asp	Val	Ile	His	Leu	Leu	Glu	Met	Leu	Cys	
			405					410					415			
GAA	GAC	TAT	GGA	GGG	AGA	GTG	GAT	TTG	GCA	AAG	GCA	TAT	TAC	AAA	GCA	1354
Glu	Asp	Tyr	Gly	Gly	Arg	Val	Asp	Leu	Ala	Lys	Ala	Tyr	Tyr	Lys	Ala	
		420					425					430				
ATG	ACC	AAA	TCA	ATA	AAT	AAA	CAT	TTT	AAA	GGA	AAT	GGA	GTC	ATT	GCA	1402
Met	Thr	Lys	Ser	Ile	Asn	Lys	His	Phe	Lys	Gly	Asn	Gly	Val	Ile	Ala	
435						440					445					
AGT	ATG	GAA	CAT	TGT	AAC	GAC	TTC	ATG	TTC	CTT	GGC	ACG	GAA	GCT	ATC	1450
Ser	Met	Glu	His	Cys	Asn	Asp	Phe	Met	Phe	Leu	Gly	Thr	Glu	Ala	Ile	
450					455					460					465	
TCT	CTT	GGT	CGT	GTT	GGT	GAT	GAC	TTT	TGG	TGC	ACG	GAC	CCC	TCT	GGT	1498
Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	Asp	Pro	Ser	Gly	
				470				475						480		
GAT	CCA	AAC	GGT	ACG	TTT	TGG	CTC	CAA	GGA	TGT	CAC	ATG	GTT	CAT	TGT	1546
Asp	Pro	Asn	Gly	Thr	Phe	Trp	Leu	Gln	Gly	Cys	His	Met	Val	His	Cys	
			485					490					495			
GCC	AAC	GAC	AGC	TTG	TGG	ATG	GGG	AAC	TTC	ATC	CAC	CCT	GAC	TGG	GAT	1594
Ala	Asn	Asp	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	His	Pro	Asp	Trp	Asp	
		500					505					510				
ATG	TTC	CAA	TCC	ACC	CAC	CCT	TGT	GCC	GCC	TTC	CAT	GCT	GCC	TCT	CGA	1642
Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Ala	Phe	His	Ala	Ala	Ser	Arg	
	515					520					525					
GCC	ATC	TCT	GGT	GGC	CCG	ATC	TAT	GTT	AGT	GAT	TCT	GTG	GGA	AAG	CAT	1690
Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Val	Ser	Asp	Ser	Val	Gly	Lys	His	
530					535					540					545	
AAC	TTT	GAT	CTT	CTG	AAA	AAA	CTA	GTG	CTT	CCT	GAT	GGA	TCG	ATC	CTT	1738
Asn	Phe	Asp	Leu	Leu	Lys	Lys	Leu	Val	Leu	Pro	Asp	Gly	Ser	Ile	Leu	
				550					555					560		
CGA	AGT	GAG	TAC	TAT	GCA	CTC	CCG	ACT	CGC	GAT	TGT	TTG	TTT	GAA	GAC	1786
Arg	Ser	Glu	Tyr	Tyr	Ala	Leu	Pro	Thr	Arg	Asp	Cys	Leu	Phe	Glu	Asp	
			565					570					575			
CCT	TTG	CAT	AAT	GGA	GAA	ACT	ATG	CTT	AAG	ATT	TGG	AAT	CTC	AAC	AAG	1834
Pro	Leu	His	Asn	Gly	Glu	Thr	Met	Leu	Lys	Ile	Trp	Asn	Leu	Asn	Lys	
		580					585					590				
TTC	ACT	GGA	GTG	ATT	GGT	GCA	TTC	AAC	TGC	CAA	GGA	GGA	GGA	TGG	TGT	1882
Phe	Thr	Gly	Val	Ile	Gly	Ala	Phe	Asn	Cys	Gln	Gly	Gly	Gly	Trp	Cys	
	595					600					605					
CGT	GAG	ACA	CGC	CGC	AAC	CAA	TGC	TTT	TCA	CAA	TAC	TCA	AAA	CGA	GTG	1930

Arg	Glu	Thr	Arg	Arg	Asn	Gln	Cys	Phe	Ser	Gln	Tyr	Ser	Lys	Arg	Val		
610					615					620					625		
ACA	TCC	AAA	ACT	AAC	CCA	AAA	GAC	ATA	GAA	TGG	CAC	AGT	GGA	GAA	AAC	1978	
Thr	Ser	Lys	Thr	Asn	Pro	Lys	Asp	Ile	Glu	Trp	His	Ser	Gly	Glu	Asn		
				630					635					640			
CCT	ATC	TCT	ATT	GAA	GGC	GTT	AAA	ACC	TTT	GCG	CTT	TAC	CTC	TAT	CAA	2026	
Pro	Ile	Ser	Ile	Glu	Gly	Val	Lys	Thr	Phe	Ala	Leu	Tyr	Leu	Tyr	Gln		
			645					650					655				
GCC	AAA	AAA	CTT	ATC	CTC	TCC	AAG	CCC	TCT	CAA	GAT	CTT	GAC	ATA	GCT	2074	
Ala	Lys	Lys	Leu	Ile	Leu	Ser	Lys	Pro	Ser	Gln	Asp	Leu	Asp	Ile	Ala		
		660					665					670					
CTT	GAC	CCA	TTC	GAA	TTC	GAG	CTC	ATC	ACT	GTT	TCA	CCA	GTG	ACC	AAA	2122	
Leu	Asp	Pro	Phe	Glu	Phe	Glu	Leu	Ile	Thr	Val	Ser	Pro	Val	Thr	Lys		
	675					680					685						
CTC	ATC	CAA	ACT	TCT	CTA	CAC	TTT	GCC	CCA	ATT	GGG	CTG	GTG	AAC	ATG	2170	
Leu	Ile	Gln	Thr	Ser	Leu	His	Phe	Ala	Pro	Ile	Gly	Leu	Val	Asn	Met		
690				695				700						705			
CTT	AAC	ACT	AGT	GGA	GCC	ATC	CAA	TCT	GTG	GAC	TAT	GAC	GAT	GAC	CTA	2218	
Leu	Asn	Thr	Ser	Gly	Ala	Ile	Gln	Ser	Val	Asp	Tyr	Asp	Asp	Asp	Leu		
				710				715					720				
AGC	TCA	GTC	GAG	ATT	GGT	GTC	AAA	GGG	TGT	GGT	GAG	ATG	CGA	GTA	TTT	2266	
Ser	Ser	Val	Glu	Ile	Gly	Val	Lys	Gly	Cys	Gly	Glu	Met	Arg	Val	Phe		
		725					730					735					
GCA	TCG	AAA	AAA	CCA	AGG	GCT	TGT	CGT	ATT	GAT	GGG	GAG	GAT	GTT	GGG	2314	
Ala	Ser	Lys	Lys	Pro	Arg	Ala	Cys	Arg	Ile	Asp	Gly	Glu	Asp	Val	Gly		
		740					745					750					
TTC	AAG	TAT	GAT	CAG	GAC	CAA	ATG	GTG	GTG	GTT	CAA	GTG	CCA	TGG	CCA	2362	
Phe	Lys	Tyr	Asp	Gln	Asp	Gln	Met	Val	Val	Val	Gln	Val	Pro	Trp	Pro		
	755					760					765						
ATT	GAT	TCT	TCA	TCG	GGT	GGC	ATT	TCG	GTT	ATC	GAG	TAC	TTG	TTT		2407	
Ile	Asp	Ser	Ser	Ser	Gly	Gly	Ile	Ser	Val	Ile	Glu	Tyr	Leu	Phe			
770					775					780							
TAATTTTAT	TTATGTAAGC	TCAATGATTG	TTGTTGTTGT	CGCTGTTGTT	GCTATCAATG	2467											
TATTTCTCTC	CAAAAGAAAA	TTATGTGTAA	TTTGGAGAGT	AATTAAGTGA	2517												

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 784 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Ala	Pro	Ser	Phe	Lys	Asn	Gly	Gly	Ser	Asn	Val	Val	Ser	Phe	Asp		
1				5				10						15			
Gly	Leu	Asn	Asp	Met	Ser	Ser	Pro	Phe	Ala	Ile	Asp	Gly	Ser	Asp	Phe		
			20					25				30					
Thr	Val	Asn	Gly	His	Ser	Phe	Leu	Ser	Asp	Val	Pro	Glu	Asn	Ile	Val		
		35					40					45					
Ala	Ser	Pro	Ser	Pro	Tyr	Thr	Ser	Ile	Asp	Lys	Ser	Pro	Val	Ser	Val		

50	55	60
Gly Cys Phe Val Gly Phe Asp Ala Ser Glu Pro Asp Ser Arg His Val		
65	70	75
Val Ser Ile Gly Lys Leu Lys Asp Ile Arg Phe Met Ser Ile Phe Arg		80
85	90	95
Phe Lys Val Trp Trp Thr Thr His Trp Val Gly Arg Asn Gly Gly Asp		
100	105	110
Leu Glu Ser Glu Thr Gln Ile Val Ile Leu Glu Lys Ser Asp Ser Gly		
115	120	125
Arg Pro Tyr Val Phe Leu Leu Pro Ile Val Glu Gly Pro Phe Arg Thr		
130	135	140
Ser Ile Gln Pro Gly Asp Asp Asp Phe Val Asp Val Cys Val Glu Ser		
145	150	155
Gly Ser Ser Lys Val Val Asp Ala Ser Phe Arg Ser Met Leu Tyr Leu		
165	170	175
His Ala Gly Asp Asp Pro Phe Ala Leu Val Lys Glu Ala Met Lys Ile		
180	185	190
Val Arg Thr His Leu Gly Thr Phe Arg Leu Leu Glu Glu Lys Thr Pro		
195	200	205
Pro Gly Ile Val Asp Lys Phe Gly Trp Cys Thr Trp Asp Ala Phe Tyr		
210	215	220
Leu Thr Val His Pro Gln Gly Val Ile Glu Gly Val Arg His Leu Val		
225	230	235
Asp Gly Gly Cys Pro Pro Gly Leu Val Leu Ile Asp Asp Gly Trp Gln		
245	250	255
Ser Ile Gly His Asp Ser Asp Pro Ile Thr Lys Glu Gly Met Asn Gln		
260	265	270
Thr Val Ala Gly Glu Gln Met Pro Cys Arg Leu Leu Lys Phe Gln Glu		
275	280	285
Asn Tyr Lys Phe Arg Asp Tyr Val Asn Pro Lys Ala Thr Gly Pro Arg		
290	295	300
Ala Gly Gln Lys Gly Met Lys Ala Phe Ile Asp Glu Leu Lys Gly Glu		
305	310	315
Phe Lys Thr Val Glu His Val Tyr Val Trp His Ala Leu Cys Gly Tyr		
325	330	335
Trp Gly Gly Leu Arg Pro Gln Val Pro Gly Leu Pro Glu Ala Arg Val		
340	345	350
Ile Gln Pro Val Leu Ser Pro Gly Leu Gln Met Thr Met Glu Asp Leu		
355	360	365
Ala Val Asp Lys Ile Val Leu His Lys Val Gly Leu Val Pro Pro Glu		
370	375	380
Lys Ala Glu Glu Met Tyr Glu Gly Leu His Ala His Leu Glu Lys Val		
385	390	395
Gly Ile Asp Gly Val Lys Ile Asp Val Ile His Leu Leu Glu Met Leu		
405	410	415
Cys Glu Asp Tyr Gly Gly Arg Val Asp Leu Ala Lys Ala Tyr Tyr Lys		
420	425	430
Ala Met Thr Lys Ser Ile Asn Lys His Phe Lys Gly Asn Gly Val Ile		
435	440	445

Ala	Ser	Met	Glu	His	Cys	Asn	Asp	Phe	Met	Phe	Leu	Gly	Thr	Glu	Ala	450	455	460
Ile	Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	Asp	Pro	Ser	465	470	475
Gly	Asp	Pro	Asn	Gly	Thr	Phe	Trp	Leu	Gln	Gly	Cys	His	Met	Val	His	485	490	495
Cys	Ala	Asn	Asp	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	His	Pro	Asp	Trp	500	505	510
Asp	Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Ala	Phe	His	Ala	Ala	Ser	515	520	525
Arg	Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Val	Ser	Asp	Ser	Val	Gly	Lys	530	535	540
His	Asn	Phe	Asp	Leu	Leu	Lys	Lys	Leu	Val	Leu	Pro	Asp	Gly	Ser	Ile	545	550	555
Leu	Arg	Ser	Glu	Tyr	Tyr	Ala	Leu	Pro	Thr	Arg	Asp	Cys	Leu	Phe	Glu	565	570	575
Asp	Pro	Leu	His	Asn	Gly	Glu	Thr	Met	Leu	Lys	Ile	Trp	Asn	Leu	Asn	580	585	590
Lys	Phe	Thr	Gly	Val	Ile	Gly	Ala	Phe	Asn	Cys	Gln	Gly	Gly	Gly	Trp	595	600	605
Cys	Arg	Glu	Thr	Arg	Arg	Asn	Gln	Cys	Phe	Ser	Gln	Tyr	Ser	Lys	Arg	610	615	620
Val	Thr	Ser	Lys	Thr	Asn	Pro	Lys	Asp	Ile	Glu	Trp	His	Ser	Gly	Glu	625	630	635
Asn	Pro	Ile	Ser	Ile	Glu	Gly	Val	Lys	Thr	Phe	Ala	Leu	Tyr	Leu	Tyr	645	650	655
Gln	Ala	Lys	Lys	Leu	Ile	Leu	Ser	Lys	Pro	Ser	Gln	Asp	Leu	Asp	Ile	660	665	670
Ala	Leu	Asp	Pro	Phe	Glu	Phe	Glu	Leu	Ile	Thr	Val	Ser	Pro	Val	Thr	675	680	685
Lys	Leu	Ile	Gln	Thr	Ser	Leu	His	Phe	Ala	Pro	Ile	Gly	Leu	Val	Asn	690	695	700
Met	Leu	Asn	Thr	Ser	Gly	Ala	Ile	Gln	Ser	Val	Asp	Tyr	Asp	Asp	Asp	705	710	715
Leu	Ser	Ser	Val	Glu	Ile	Gly	Val	Lys	Gly	Cys	Gly	Glu	Met	Arg	Val	725	730	735
Phe	Ala	Ser	Lys	Lys	Pro	Arg	Ala	Cys	Arg	Ile	Asp	Gly	Glu	Asp	Val	740	745	750
Gly	Phe	Lys	Tyr	Asp	Gln	Asp	Gln	Met	Val	Val	Val	Gln	Val	Pro	Trp	755	760	765
Pro	Ile	Asp	Ser	Ser	Ser	Gly	Gly	Ile	Ser	Val	Ile	Glu	Tyr	Leu	Phe	770	775	780

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc= "Synthetic DNA"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTYTAYCTBA CHGTNCAAYCC TCA

23

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc= "Synthetic DNA"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTYTAYCTBA CHGTNCAAYCC CCA

23

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc= "Synthetic DNA"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTYTAYCTBA CHGTNCAAYCC ACA

23

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc= "Synthetic DNA"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTYTAYCTBA CHGTNCAAYCC GCA

23

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc= "Synthetic DNA"
- (ix) FEATURE:
  - (A) NAME/KEY:
  - (B) LOCATION:

(D) OTHER INFORMATION: N at 6 and 11 = inosine  
Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GARGGNGTNN GNCACTGTGT NGAYGG

26

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (D) OTHER INFORMATION: N at 6 and 11 = inosine  
Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GARGGNGTNN GNCACTGTGT NGAYGG

26

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (D) OTHER INFORMATION: N at 6 and 11 = inosine  
Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GARGGNGTNN GNCACTTGTGT NGAYGG

26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (D) OTHER INFORMATION: N at 3 = inosine

Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

26

GTNGGNTGYT TYGTNGGYTT YGAYGC

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION: N at 3 = inosine

Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

26

GTNGGNTGYT TYGTNGGRTT YGAYGC

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION: N at 9 and 11 = inosine

Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

29

TTYGAYGCNT CNGARCCHGA YTCDCGNCA

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc= "Synthetic DNA"

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION: N at 9 and 11 = inosine

Other N = A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:  
TTYGAYGCNT CNGARCCHGA YTCDAGYCAY

30

(2) INFORMATION FOR SEQ ID NO:17:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
    (A) DESCRIPTION: /desc= "Synthetic DNA"  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:  
GAYCARGAYC TRATGGTNGT

20

(2) INFORMATION FOR SEQ ID NO:18:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 26 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
    (A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
    (A) NAME/KEY:  
    (B) LOCATION:  
    (D) OTHER INFORMATION: N at 6 and 15 = inosine  
                                    Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:  
CCRTCACACG GRTGNCKNAC NCCYTC

26

(2) INFORMATION FOR SEQ ID NO:19:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 26 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
    (A) DESCRIPTION: /desc= "Synthetic DNA"  
(ix) FEATURE:  
    (A) NAME/KEY:  
    (B) LOCATION:  
    (D) OTHER INFORMATION: N at 6 and 15 = inosine  
                                    Other N = A, G, C, or T  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:  
CCRTCACACG GRTGNCKNAC NCCYTC

26

(2) INFORMATION FOR SEQ ID NO:20:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: other nucleic acid  
 (A) DESCRIPTION: /desc= "Synthetic DNA"  
 (ix) FEATURE:  
 (A) NAME/KEY:  
 (B) LOCATION:  
 (D) OTHER INFORMATION: N at 6 and 15 = inosine  
 Other N = A, G, C, or T  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:  
 CCRTCACAYA TRTGNCCKNAC NCCYTC

26

(2) INFORMATION FOR SEQ ID NO:21:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: other nucleic acid  
 (A) DESCRIPTION: /desc= "Synthetic DNA"  
 (ix) FEATURE:  
 (A) NAME/KEY:  
 (B) LOCATION:  
 (D) OTHER INFORMATION: N at 3 and 18 = inosine  
 Other N = A, G, C, or T  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:  
 TGNCCHGART CDGGYTCNGA NGCRTCRAA

29

(2) INFORMATION FOR SEQ ID NO:22:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: other nucleic acid  
 (A) DESCRIPTION: /desc= "Synthetic DNA"  
 (ix) FEATURE:  
 (A) NAME/KEY:  
 (B) LOCATION:  
 (D) OTHER INFORMATION: N at 19 = inosine  
 Other N = A, G, C, or T  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:  
 RTGRCTHGAR TCDGGYTCNG ANGRTCRAA

30